Proceedings of the 30th Annual Aquatic Toxicity Workshop: September 28 to October 1, 2003, Ottawa, Ontario

Comptes rendus du 30e atelier annuel sur la toxicité aquatique: du 28 septembre au 1er october 2003, Ottawa (Ontario)

Editors/Éditeurs

K. Hedley[1], S. Roe[2] and/et A.J. Niimi[3]

[1]Environment Canada, National Water Research Institute, Gatineau, QC, K1A 0H3
[2]Environment Canada, National Guidelines and Standards Office, Gatineau, QC, K1A 0H3
[3]Department of Fisheries and Oceans, Bayfi[illegible] Institute, Burlington, ON, L7R 4A6

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Preface / Preface

The 30th Annual Aquatic Toxicity Workshop was held at the Crowne Plaza Ottawa Hotel in Ottawa, Ontario, September 28 to October 1, 2003. The Workshop included 5 plenary presentations, 123 platform and 53 poster papers. Total attendance was 357.

This Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are administered by a Board of Directors, and organized by local organizing committees. The Proceedings are published with the support of the Department of Fisheries and Oceans.

L' 30e atelier annuel sur la toxicité a eu lieu Hôtel Crowne Plaza d'Ottawa, Ottawa (Ontario), 28 octobre au 1er october 2003. Le atelier a donné lieu a 5 communication lors de séances plénières, 123 exposés d'invités d'honneur 53 communications par affichage. 357 personnes ont assisté au atelier.

Le atelier a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

Editors comments / Remarques des editeurs

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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Table of Contents/Tables des matiére

Page

Plenary/Plénière

Bridge over troubled water: water issues over the past 30 years/Bâtir des ponts eneaux troubles: trente ans de questions environnementales

Linking aquatic science and policy. K. Brown. 1
Recent advances in freshwater research. D.R.S. Lean. 1
Fifty ways to clean your river: the pulp and paper industry in the St. Lawrence River. J.-P. De Serres, G. Martin and A. Lo. 1
A new approach to water management. R. Findlay.
Safeguarding Canada's freshwater resources: international aspects. A.L. Hamilton 10
10

Contributed Papers/Documents contribués

Building a stronger bridge between laboratory and field/Bâtir un pont solide entre le laboratoire et le terrain

Algae, cyanobacteria and aquatic macrophytes for ecotoxicity tests: maintenance and sources of axenic cultures. J.C. Acreman. (PL)* 12
Can benthic invertebrate communities directly affect laboratory toxicity test organisms in sediment quality studies? M.D. Paine, E. DeBlois, S.A. Whiteway, U. Williams and F. Power. (PL) 13
Toxicity and bioaccumulation of tributyltin (TBT) from freshwater harbour sediments. A.J. Bartlett, D.G. Dixon, R.J. Maguire, S.P. Batchelor and U. Borgmann. (PL) 13
Using tissue residue effects as an ecotoxicological framework to establish links between laboratory and field studies. M.H. Salazar. (PL) 14

De-mystifying mixtures/La démystification des mélanges

Modelling multiple metal binding to fish gills and to natural organic matter. R.C. Playle, S. Smith and A. Winter. (PL) 14
Concentration addition vs effects addition models: evaluation of 10-metal mixture toxicity. W.P. Norwood, D.G. Dixon and U. Borgmann. (PL) 15
Synergistic toxicity of mixtures: metal/PAH/oxyPAHs impacts via catalytic generation of reactive oxygen species (ROS). B.M. Greenberg, X.D. Huang, T.S. Babu, M. Lampi, J. Nykamp and F. Xie. (PL) 15
Why does crude oil cause blue sac disease in larval fish? P.V. Hodson, C. Noble, H. Orr, P. Quereshi, S.P. Tabash and M. Bauder. (PL) 15
Toxicity of oil sands to early developmental stages of fathead minnows (*Pimephales promelas*). M.V. Colavecchia, S. Backus, P.V. Hodson and J.L. Parrott. (PL) 16
Histopathological effects of naphthenic acids and salinity to goldfish (*Carassius auratus*). V. Nero, A.J. Farwell, A. Lister, L.E.J. Lee, G.J. Van Der Kraak and D.G. Dixon. (PL) 16
Comparative toxicity of water-accommodated fractions of oil and dispersed oil to marine fish larvae. C.M. Couillard, K. Lee, B. Legare and S. St. Pierre. (PL) 16
Pulp mill biosolids: an initial survey of wood extractive constituents and aquatic toxicity. S. Hawkins C. Hedley, M.R. van den Heuvel and P.V. Hodson. (PL) 17
Resin acid biotransformation in two pulp mill biosolids. S. Hawkins, C.W. Khan and M.R. van den Heuvel. (PL) 17

---

*PL:Platform paper/Présentation orale. PO: Poster paper/Présentation affichée. IPP:Interactive poster papers/Présentations interactive affichée

Blue sac disease in early life stages of white suckers (*Catastomus commersoni*) exposed to oil sands. M.V. Colavecchia, P.V. Hodson and J.L. Parrott. (PO) 18

A special challenge for the Domestic Substances List program: categorizing "unknown or variable composition, complex reaction products and biological materials" (UVCBs). S. Schnabel, D. Caron, P. Doyle, P. Robinson, A. Sene, A. Okonski and A. Au. (PO) 18

CYP1A inducing compounds in crude oil. C.W. Khan, B. Hollebone, S. Ramachandran, L.M. Clarke, Z. Wang and P.V. Hodson. (PO) 19

Environmental Effects Monitoring (EEM)/Étude de suivi des effet sur l'environnement (ESEE) EEM-pulp and paper, "The unabridged version"/ESEE - pâtes et papiers, "La version intégrale" 19

10 Years of EEM for pulp and paper – we've come a long way! K. Hedley. (PL) 19

A 10 year industry retrospective on the EEM program. R. Cook and I. Cloutier. (PL) 20

Environmental effects of pulp and paper mill effluents in Canada following regulatory changes made in 1992. M.E. McMaster, J.L. Parrott and L.M. Hewitt. (PL)

Tracking the outcome of environmental investments in the 1990s: a case study. P.H. Martel, T.G. Kovacs and R. Voss. (PL) 20

Reducing uncertainties in pulp and paper EEM by using mesocosms and caged bivalves. M.H. Salazar and S.M. Salazar. (PL) 21

A comparison of the fish survey and caged bivalve approach for pulp and paper EEM. S.D. St. Jean, S.C. Courtenay and W.R. Parker. (PL) 21

Advances in study designs and measurement endpoints used in the environmental effects monitoring program. R.B. Lowell and J.M. Culp. (PL) 22

Development for investigation of cause in pulp and paper environmental effects monitoring. L.M. Hewitt, M.G. Dubé, S. Ribey, K. Hedley, J.M. Culp, D.L. MacLatchy and K.R. Munkittrick. (PL) 22

Assessing effects of pulp and paper mill effluent using a modified fathead minnow (*Pimephales promelas*) bioassay. C.J. Rickwood, M.G. Dubé, D. MacLatchy, J.L. Parrott and L.M. Hewitt. (PO) 22

Gonadosomatic index for caged blue mussels (*Mytilus spp.*): Pacific and Atlantic comparison. S.D. St. Jean, P. van Pollelen, K.K.J. Kim, F. Bishay and S.C. Courtenay. (PO) 23

Proteomic profiling of abietic acid exposure in rainbow trout (*Oncorhynchus mykiss*) cell lines. L.E.J. Lee, S.K. Wagg, S.B. Willfang, P. Ibrahim and M.S. Allen. (PO) 23

Environmental effects monitoring (EEM) of pulp and paper mills in Quebec: summary of toxicity for the period 2000-2003 (six first series of Cycle 3). M. Mikhail, I. Matteau and C. Langlois. (PO) 24

EEM - metal mining/ESEE - mines de métaux

Baseline EEM program for the Tulsequah Chief Mine - northwestern British Columbia. D. Osmond and F. Pearson. (PL) 24

Metals and metallothionein in fish from a freshwater system receiving gold mining effluents. V.P. Palace, C.L. Baron, R.E. Evans, S. Kollar, J. Werner and K.L. Wautier. (PL) 25

The effects of metal ion exposure on α-Synuclein aggregation in the nervous system of fish. G. Ross, H. Ross, J. Rossiter, L. Williams and V.P. Palace. (PL) 25

Comparison of a partial life cycle bioassay in artificial streams to a standard beaker bioassay to assess metal mine effects on *Chironomus tentans*. K.A. Hruska, M.G. Dubé and G. Watson. (PL) 25

Acute toxicity of sulfur oxyanions to *Daphnia magna* and *Selenastrum capricornutum*. M.L. Schwartz, J.C. McGeer, M.M. King, S. Brigham and J.C. Nadeau. (PL) 26

Aquatic moss (*Fontinalis antipyretica*) 21 day growth bioassay. J. Pickard and T. Davies. (PL) 26

Culturing of metal mining EEM toxicity testing organisms. C.A. Huras. (PL) 26

Repeated measures (RM) ANOVA and regression designs for environmental effects monitoring programs. M.D. Paine. (PL) 27

Using artificial streams to assess the effects of metal mine effluent effects on the lifecycle of the freshwater midge (*Chironomus tentans*). K.A. Hruska, M.G. Dubé and G. Watson. (PO) 27

Use of small bodied fish species in EEM programs: benefits and costs of age determination. P. Orr, C. Russel, J. Tost and R. Pedlar. (PO) 27

Ecological risk assessment of metals in the environment/Évaluation des risques écologiques des métaux dans l'environnement

Metal sources for freshwater invertebrates: pertinence for risk assessment. L. Hare et A. Tessier. (PL) 28

Using bivalve tissue copper residues and effects for ecological risk assessment. M.H. Salazar and S.M. Salazar. (PL) 28

Bioaccumulation of metals by *Hyalella azteca*: which models best describe accumulation? W.P. Norwood, D.G. Dixon and U. Borgmann. (PL) 29

Integrating a metal bioaccumulation model into risk assessment methods. R.R. Goulet, P.J. Doyle and L. Hare. (PL) 29

The role of risk assessment in determining chemistry needs at contaminated sites. G. Gilron, R. Hull, C. Bacigalupo and G. Ferguson. (PL) 30

Management of the mercury issue at the La Grande hydroelectric complex, Québec, Canada. R. Schetagne. (PL) 30

Old fish, small lakes, and high mercury levels in lake trout (*Salvelinus namaycush*) in northern Canada. L.M. Doetzel, M.S. Evans, D.C.G. Muir, W.L. Lockhart, G. Low, J. Delaronde, J. Keating and G. Stern. (PL) 30

Mercury alters neurochemical receptor binding characteristics in wild mink (*Mustela vison*). N. Basu, M. Gamberg, K. Klenavic, D. Evans, A.M. Scheuhammer and H.M. Chan. (PL) 31

Hazard and ecological risk assessments of metals, metalloids and inorganic metal substances. P.M. Chapman, F. Wang, R.R. Goulet and C. Kamunde. (PL) 31

Geochemistry of lead and chronology of its deposition in remote Canadian shield lakes. C. Gallon, C. Gobeil, J.C. Nadeau and A. Tessier. (IPP) 32

The nature of aerosolic particulates in the Sudbury smelter footprint. G. Spiers, J. Richard, C. Hawson and A. Cheburkin. (IPP) 32

The speciation of trace metals at the soil:root interface in forest soils. F. Courchesne, P. Legrand, V. Séguin, S. Sauvé et M. Turmel. (IPP) 32

Risk associated with natural attenuation of trace metal contaminants in soils. W. Hendershot, K. Taillon, D. MacDonald and S. Sauvé. (IPP) 33

Metal competition in the freshwater environment - implications for environmental risk assessment. C.L. Chakrabarti, I.I. Fasfous, T. Yapici and J. Murimboh. (IPP) 33

Study design for a contaminated foreshore risk assessment: a weight-of-evidence approach. G. Wickstrom and M. Cameron. (IPP) 34

Development of a bioaccumulation model for a sedimentary trace metal biomonitor. L. Croisetiere, L. Hare et A. Tessier. (IPP) 34

How does research on the invertebrate *Hyalella azteca* fit into environmental risk assessment? W.P. Norwood, J. Schroeder, M. Nowierski, A. Wallace, D.G. Dixon and U. Borgmann. (IPP) 34

Influence of hydrogen ions on cadmium accumulation by members of a planktonic trophic chain. J. Orvoine, L. Hare et A. Tessier. (IPP) 35

Metal detoxification and antioxidant defences in indigenous yellow perch (*Perca flavescens*) exposed to cadmium, copper, nickel and zinc. A. Giguère, P.G.C. Campbell, L. Hare, C. Cossu-Leguille, P. Couture, G.D. McDonald and J.B. Rasmussen. (IPP) 35
Cadmium dynamics in yellow perch (*Perca flavescens*) following transplantation to a metal-contaminated lake. L. Kraemer, P.G.C. Campbell et L. Hare. (IPP) 36
Population and physiological effects of industrial metal contamination in wild yellow perch (*Perca flavescens*). P. Couture, J. Rajotte and G. Pyle. (IPP) 36
Acclimation effects of different dietary factors on metal uptake and toxicity in freshwater fish. S. Niyogi, C. Kamunde, B. Baldisserotto, M. Grosell, G.D. McDonald and C.M. Wood. (IPP) 37
Aluminum binding to fish gills and to natural organic matter. R.C. Playle, J. Nichols, A. Winter and S. Smith. (IPP) 37
Evaluation of parameters regulating phagocytosis in the rainbow trout (*Oncorhynchus mykiss*) gill cell line, RTgill-W1. D. Sotornik, N.C. Bols and L.E.J. Lee. (IPP) 37

Exposure/Effects - mechanistic models/Exposition/effets - modèles mécanistes

Progesterone levels in a key species in coastal sediments, the annelid polychaete, *Hediste diversicolor*. C. Mouneyrac, J. Pellerin, C. Durou, C. Amiard-Triquet et P. Rainbow. (PL) 38
Mechanisms of cadmium-induced adrenotoxicity in trout (*Oncorhynchus mykiss*) adrenocortical cells: involvement of calcium channels. A. Hontela and A. Lacroix. (PL) 44
Effects of hydroxylated PCBs (OH-PCBs) on thyroid status of rainbow trout (*Oncorhynchus mykiss*). A. Buckman, A.T. Fisk, K.R. Solomon and S.B. Brown. (PL) 45
Tracking DDT pathways to tree swallows (*Tachycineta bicolor*) in Point Pelee National Park. M. Sebastian, J.J.H. Ciborowski, J.E.G. Smits and G.R. Bortolotti. (PL) 45
Temperature modifications of PAH-induced CYP1A activity in fish. J. Dungavell, T. Cross, J. Ennis and P.V. Hodson. (PL) 45
Thermal modulation of the excretion of benzo[*a*]pyrene in teleosts. B.D. Johnston and C.J. Kennedy. (PL) 46
Chronic toxicity of hydrophobic compounds measured using partition controlled delivery. D.Turcotte, P. Akhtar, Y. Kiparissis, P.V. Hodson and R.S. Brown. (PL) 46
Significance of oil droplets in chemically enhanced water-accommodated fraction. S. Ramachandran, P.V. Hodson, and K. Lee. (PL) 47
Chemical profile of crude oils in support of toxicity studies. P. Akhtar and R.S. Brown. (PL) 47
The effects of cadmium on the cytoskeleton of the human hepatocellular carcinoma (HepG2) cell line. E. Diringer, E. Welnhofer and P.F. Dehn. (PL) 47
Developing rapid toxicity tests with the ciliated protozoan, *Tetrahymena thermophila*, that utilize microwell filter plates. V.R. Dayeh, D. Sotornik, D. Lynn, N.C. Bols and L.E.J. Lee. (PL) 48
Biotransformation of perfluorooctane sulfonamide and ethyl perfluorooctane sulfonamide by rainbow trout (*Oncorhynchus mykiss*) liver microsomes. V.P. Palace, G. Tomy, S. Tittlemeir, E. Braekevelt, W. Budakowski, B. Lau, J.G. Eales and J. Plohman. (PL) 48
Thyroid and vitamin A status in mink (*Mustela vison*) fed PCB-contaminated carp (*Cyprinus carpio*) from Saginaw Bay. V.P. Palace, P. Martin, S. Bursian and G. Mayne. (PL) 49
Fate of PAHs in internal organs, muscle and gall bladder bile of finfish. J.D. Leonard and J. Hellou. (PL) 49
Risk assessment and the magic duck. G. Wickstrom and M. Cameron. (PL) 52
Toxicity of naphthenic acids in tissue culture water and environmental water to fish cell lines. V.R. Dayeh, L.E.J. Lee, D.G. Dixon and N.C. Bols. (PL) 52

The toxicity of photomodified polycyclic aromatic hydrocarbons to Japanese medaka *(Oryzias latipes)* embryos. A.J. Farwell, M. Croft, S.M. Rhodes and D.G. Dixon. (PL) 52

Use of QSARs in the categorization Of discrete organic substances on Canada's Domestic Substances List. P. Robinson. (PL) 53

Experimental sediment exchanges between natural and constructed wetlands on oil sands leases, near Fort McMurray, Alberta: a look at the relationship between sediment treatments and benthic macroinvertebrates colonizers. L. Barr, J.J.H. Ciborowski, N. Cooper and L. Foote. (PL) 53

Effects/impacts - ecological effects/Effets, impact - importance écologique

Long-term survival of early life stage fish exposed to PAH-contaminated sediment. M.E. Bowerman, H.E. Tarnowski, C.W. Khan and P.V. Hodson. (PL) 54

Comparisons of metallothionein concentrations, cadmium accumulation and Gene expression of metallothionein 20IIa cDNA in gills of *Mytilus edulis* after cadmium and estradiol exposure. J. Pellerin, J.-C. Amiard et C. Gratas. (PL) 54

Bivalve population status and biomarker responses in *Mya arenaria* clams (Saguenay Fjord, Québec, Canada). F. Gagné, C. Blaise et J. Pellerin. (PL) 61

A simple method to evaluate whether a biological community has been influenced by anthropogenic activity. M.F. Bowman, K.M. Somers and R.A. Reid. (PL) 62

Defining reference and degraded conditions: integrating approaches to evaluating environment effects. J.J.H. Ciborowski, J.A. Schuldt, L.B. Johnson, G.E. Host, T. Hollenhorst and C. Richards. (PL) 72

Using criteria in a biological monitoring and assessment framework. B.W. Kilgour, K.R. Munkittrick, C. Portt, K. Hedley, J.M. Culp, S.S. Dixit and G. Pastershank. (PL) 72

Deciding how to monitor marine oil spills: implementing environmental effects monitoring during oil spills in Canada. K. Trudel. (PL) 73

Shortcomings of using provincial environmental quality guidelines in risk assessment of polycyclic aromatic hydrocarbons. G. Gilron and A. Safruk. (PL) 73

Forensic ecotoxicology – a tool for managing toxics in the environment: gas plant effluent study. D.A. Birkholz, J.S. Goudey, M. Ralitsch, J. Proczkowski and R. Brockbank. (PL) 73

The fate, behavior, and ecotoxicity of new polymers in the aquatic environment. S. Lawuyi, S. Falicki, A.J. Atkinson and G. Hammond. (PO) 74

Comparative toxicology of four crude oils. L.M. Clarke, P.V. Hodson and R.S. Brown. (PO) 74

Temporal changes in contaminants and trophic levels in biota from Yukon subarctic lakes. M.J. Ryan, G.A. Stern, K.A. Kidd, M. Diamond, M.V. Croft, S. Gewurtz and P. Roach. (PO) 74

Revisiting the 1994 St. Clair River sediment quality triad. T. Kierstead, T. Moran, M. Gardner and S. Munro. (PO) 75

Evaluation and refinement of Environment Canada's primary hepatocyte test method for measuring acute lethality of metal mining effluents. R. Chong-kit, J. Schroeder, F. Gagné, D.G. Poirier, J.A. Miller and R.P. Scroggins. (PO) 76

ETOX - database for aquatic and terrestrial ecotoxicological information. D. Schudoma. (PO) 76

The changing face of aquatic toxicology in the Ontario Provincial Government. D.G. Poirier, J. Schroeder, R. Chong-kit, T.L. Watson-Leung, M. Mueller, M. Appleton and A. Collins. (PO) 76

UV-B radiation and 4-tert-octylphenol reduce survival and induce developmental abnormalities in *Rana pipiens* tadpoles. M.C. Croteau, D. Lean and V.L. Trudeau. (PO) 77

Acute toxicity of the mosquito-control pesticide, malathion to northern leopard frog (*Rana pipiens*) tadpoles. B. Perez, N. Gallant, J. Gibson, B.D. Pauli, D. Lean and V.L. Trudeau. (PO) 77

Acute and sublethal effects of rotenone on juvenile rainbow trout (*Oncorhynchus mykiss*). W.W. Cheng. (PO) 78

Substantiation validation of "low concern polymer" criteria for the assessment of new substances under the *Canadian Environmental Protection Act*. E. Karalis, A.J. Atkinson, G. Hammond, M.J. Lapointe and T. Singer. (PO) 81

Draft follow up environmental risk assessment of chlorinated paraffins in Canada. L.S. Lander and D.C.G. Muir. (PO) 82

Ecosystem stress response: understanding effects on the benthic invertebrate community of Alberta oil-sands wetlands. C.M. Wytrykush and J.J.H. Ciborowski. (PO) 82

Monitoring degradation of oil sands constituents and foodweb dynamics in aquatic reclamation using stable isotopes. A.J. Farwell, B.J. Butler, M.D. Mackinnon and D.G. Dixon. (PO) 83

Bioaccumulation of hexachlorobenzene in the aquatic food web. D. Schudoma. (PO) 83

Review of current BSAF determinations and application in environmental assessments. T.L. Watson-Leung and J. Schroeder. (PO) 83

Adverse effects on fauna of Ami River by the effluents released by the Gorakhpur Industrial Development Authority (India). R.K. Misra and S. Misra. (PO) 84

Soil and sediment toxicity evaluation: current and prospective practice and applications/Évaluation toxicologique des sols et des sédiments: Pratiques courantes et futures et application

Priorities for research and standardization of toxicology methods for assessing contaminant mixtures in Canadian soils. R.P. Scroggins, L. Novak, K. Becker-van Slooten, G.L. Stephenson, S. Siciliano, R. Kuperman and D.A. Bright. (PL) 91

Must individuals used in soil invertebrate toxicity tests originate from synchronous cultures? J.T. Crumb, G.L. Stephenson, N.C. Feisthauer, J.A. Miller, D. McLeay and R.P. Scroggins. (PL) 91

Comparison of experimental designs of earthworm (*Eisenia andrei*) reproduction tests recommended by Environment Canada and the OECD. K.D. Schneider, J.T. Crumb, G.L. Stephenson, P.Sibley and R.P. Scroggins. (PL) 92

Toxicological comparison of *Eisenia andrei* in varying soil test systems recommended by Environment Canada. J.I. Princz and R.P. Scroggins. (PL) 92

Re-evaluation of the ecotoxicity values for CCME petroleum fraction 3: should fraction 3 be split? J.H. McCann, G.L. Stephenson, J.L. Roy and D.G. Dixon. (PL) 93

Acute and chronic toxicity of the new explosive CL-20 in the earthworm (*Eisenia andrei*). P.Y. Robidoux, G.I. Sunahara, K. Savard, Y. Berthelot, S. Dodard, M. Sarrazin and J. Hawari. (PL) 93

Toxic effects of polynitro-organic compounds on enchytraeids (Oligochaeta) in freshly amended natural soils. S. Dodard, Hawari, M. Sarrazin, P. Gong, L. Paquet, G. Ampleman, S. Thiboutot and G.I. Sunahara. (PL) 93

Duckweed, collembols and more - application of effective population growth tests in soil ecotoxicity testing. M.F. Eberius. (PL) 94

Kinetics and mechanisms of PAHs sequestration in marine and freshwater sediments. D. Brion et É. Pelletier. (PL) 94

Uranium in soil – an example with very skewed effect concentrations. S. Sheppard and G.L. Stephenson. (PL) 106

Framework foundation and guidance for Tier 2 site-specific soil contact standards for PHC-contaminated sites. G.L. Stephenson, N.C. Feisthauer, M. Gilbertson and J.H. McCann. (PL) 107

Testing framework for assessing soils as part of a site-specific (ecological) risk assessment. G.L. Stephenson, N.C. Feisthauer and M. Gilbertson. (PL) 107

Valeurs de référence écotoxicologiques : du nouveau. A. Lafortune, A. Renoux, L. Martel, R. Chassé et J. Trépanier. (PL) 108
Estimation of risk from bioassay data using the TerraSys software. A. Renoux et J. Trépanier. (PL) 108
The Sudbury soils study: a case study of using soil chemistry in ecological risk assessment. C.D. Wren, M. Butler, G.M. Ferguson, R.N. Hull and G.D. Watson. (PL) 108
Predicting the fate of fungi released into the environment using laboratory-contained intact soil-core microcosms. M. Providenti, S. Mautner, M. Smith, O. Chaudry, M. Bombardier, R.P. Scroggins and E. Gregorich. (PL) 109
The effect of high and low dissolved oxgen on the toxicity of oil sands coke and its leachate to *Chironomus tentans*. A.J. Squires and K. Liber. (PO) 109
Method Summary of an echinoid embryo developmental test for determining the toxicity of sediment pore water. C. Buday. (PO) 110
A new tool for ecotoxicological risk assessment of contaminated sites: the TerraSys software. A. Renoux, J. Trépanier, P. Wallis, É. Richer, M. Cyr, M. Bisson, K. Morin et M. Fouchécourt. (PO) 110

Source to Tap - Tap to Sewer/De la source au robinet - du robinet aux égouts

Source water protection – it just makes sense, that's why! P.B. Jiapizian. (PL) 110
The application of the CCME WQI as a performance measurement tool to assess the effectiveness of best management practices R. Paterson, A. Ali Khan and H. Khan. (PL) 111
National Water Research Institute contributions in identifying threats to water quality (and enhancing the dialogue between scientists and decision-makers). A.T. Bielak. (PL) 111
Cyanobacteria: the MBA to protecting the ecosystem and human health. T. Phommavong, M. Giddings, T. Macaulay and M. Hilderman. (PL) 112
Watershed management and environmental quality objectives. H. Khan and P.B. Jiapizian. (PL) 112
Probing the Ramsey Lake watershed. G.A. Spiers, A. Lock, D.A. Pearson, B. Hostetler and J. Ray. (PL) 113
A fibre-optic probe for remote detection of *Eschericia coli* and total coliform bacteria in water. R.S. Brown, E.J. Marcotte, E. Lee, A. Ley, C. Gilmour, M. Brown, P. Aston, G. Cairns and J. Poland. (PL) 113
Designing the best strategy to manage municipal wastewater effluents. D. Ellison and C. Jefferson. (PL) 113
Metal bioaccumulation in caged mussels exposed to a municipal wastewater effluent. C. Gagnon, P. Turcotte, F. Gagné, C. Blaise and M.H. Salazar. (PL) 114

Management of effluents from municipal waste water treatment facilities in Canada/Gestion des effluents des stations municipals de traitement des eaux usées au Canada

The management of municipal wastewater effluents in Canada is changing - (the federal view). J.D. Clarke and B.A. Munson. (PL) 115
A science based approach for the management of municipal wastewater discharges. C.B. Larose and L.A. Taylor. (PL) 115
Derivation of a site specific ammonia limit for municipal treatment plant effluents. G.R. Craig and I. Middelraad. (PO) 115
Redefining regulatory terms to develop rapid response action capability to protect ecosystems from biological contamination. A.J. Niimi and V.G. Thomas. (PO) 116
Microbial pollution: a key factor to consider in the management of municipal wastewater effluents from the "smallest" of sewage outfalls. J.F. Payne, L.L. Fancey, L. Park, C. Andrews, S.A. Whiteway and B. French. (PO) 116

Fate and effects of pharmaceuticals and EDCs: Why the headache?/L'ABC des substances pertubatrices du système endocrinien et des produits pharmaceutiques

Fish, frogs and pharmaceuticals in the aquatic environment. V.L. Trudeau, T.W. Moon and C.D. Metcalfe. (PL) 117

Do pharmaceuticals in the environment get into non-target aquatic species? C. Mimeault, V.L. Trudeau, C.D. Metcalfe and T.W. Moon. (PL) 117

QSAR based toxicity ranking and prioritization of ~3000 pharmaceuticals. H. Sanderson, K.R. Solomon, R. Brain, D.J. Johnson, C.J. Wilson and T. Reitsma. (PL) 117

The tributyltin: a potential sex hormone disruptor in a *Mya arenaria* study in mesocosms. A. Siah, J.C. Pellerin, R. Saint-Louis, J. Amiard et É. Pelletier. (PL) 118

Development of a bioassay to assess lethal and sub-lethal effects of pharmaceuticals on *Hydra attenuata*. B.M. Quinn et C. Blaise. (PL) 127

Microcosm and laboratory studies into the effects of pharmaceuticals on freshwater plankton communities. C.J. Wilson, D.J. Johnson, H. Sanderson, K.R. Solomon and R. Brain. (PL) 127

Feminization of the FLFII strain of Japanese medaka (*Oryzias latipes*) exposed to 17ß-estradiol. G.C. Balch, P.A. Wilson and C.D. Metcalfe. (PL) 128

In vivo responses of rainbow trout (*Oncorhynchus mykiss*) exposed to two laundry detergent additives. D.B. Simmons, S. O'Toole, G.C. Balch and C.D. Metcalfe. (PL) 128

Pharmaceuticals in Canadian sewage treatment plant (STP) effluents: can they lead to reproductive impairment in non-target species? A.J. Woodhouse, T.W. Moon, V.L. Trudeau and G.J. Van Der Kraak. (PL) 128

Determination of *in vivo* endocrine effects in fish from nonylphenol ethoxylates and their biodegradation metabolites. G.C. Balch, C.M. Foran, B. Peterson, E. Mihaich and C.D. Metcalfe. (PL) 129

In vivo estrogenicity of nonylphenol, nonylphenol-1-ethoxylate and nonylphenol-1-ethoxycarboxylate using vitellogenin induction in juvenile rainbow trout (*Oncorhynchus mykiss*): a re-evaluation of nonylphenol and its ethoxylates in the Canadian environment. É.B. Dussault, J.P. Sherry, H.-B. Lee, B.K. Burnison and M.R. Servos. (PL) 129

An ELISA for cutthroat trout (*Oncorhynchus clarki*) vitellogenin (Vg) and its use to assess the estrogenic impacts of agricultural runoff in a British Columbia watershed. J.P. Sherry, T. Hooey, S. Sylvestre, M. Sekela, T. Tuominen, P.D. Hansen and B. Hock. (PL) 130

Assessing effects of pharmaceuticals on the aquatic environment in Canada: overview of approaches and assessment tools. K.R. Solomon, H. Sanderson, P. Sibley and S.A. Mabury. (PL) 130

The use of genetic markers of frog metamorphosis for the detection of disruption of thyroid hormone action by acetochlor and sewage treatment plant effluent. C.C. Helbing, F. Zhang, L. Ji, N. Veldhoen, K. Ovaska and G.C. van Aggelen. (PL) 131

Effects of xenoestrogens in the St. Lawrence River on male spottail shiner (*Notropis hudsonius*) reproduction. D.G. Cyr, J. Aravindakshan et V. Paquet. (PL) 131

Assessment of wild fish from some Canadian areas of concern for reproductive alterations. M.E. McMaster, G.R. Tetreault, C. Boyko and T. Janoscik. (PL) 132

An accumulation model for investigating active substances bioavailable to fish exposed to pulp mill effluents. L.M. Hewitt, A. Pryce, R. Schryer, B.K. Firth, A. Belknap, K.R. Munkittrick and G.J. Van Der Kraak. (PL) 132

Responses of a freshwater food web to synthetic estrogen additions. K.A. Kidd, C.L. Podemski, M.J. Paterson, A.G. Salki, D.L. Findlay, V.P. Palace, P.J. Blanchfield, K.H. Mills, K. Liber, M.E. McMaster, R.E. Evans and B.J. Park. (PL) 132

Short-term exposure to 17 α-ethynylestradiol alters normal development of testes in *Xenopus laevis*. S.M. Ruby, E. McKinley, C. Dimacacos and M. Fournier. (PO) 133

Toxicity testing of a polybrominated diphenyl ether (PBDE) commercial flame retardant using the *Xenopus* tail regression model. G.C. Balch, S. McDowell, K. Reiber and C.D. Metcalfe. (PO) 133

Alkylphenols, estrogenicity and androgenicity in the Miramichi River, New Brunswick. D.T. Bennie, B.K. Burnison, C.A. Sullivan, S.B. Brown and W.L. Fairchild. (PO) 134

Effect of toxaphene on the liver of female yellowtail flounder (*Limanda ferruginea* Storer). G.E. Fåhræus Van Ree and H.A. Baikie. (PO) 134

An assessment of ambient water estrogenicity at sites along the St. Clair River. J.P. Sherry, C. Tinson, K. Cooper, M.E. McMaster, S.B. Brown, J.L. Parrott, T. Moran, T. Kierstead and S. Munro. (PO) 134

"Fishy" business (agriculture)/Histoire d'eaux sales (agriculture)

Evaluation of hormonal activities in municipal biosolids and manures. A. Lorenzen and E. Topp. (PL) 135

Fate of selected endocrine-disrupting substances in agricultural soils. E. Topp, A. Lorenzen, B.K. Burnison and M.R. Servos. (PL) 135

Slimy sculpin (*Cottus cognatus*) population responses in agricultural regions of northwestern New Brunswick. M.A. Gray and K.R. Munkittrick. (PL) 136

Relative risk ranking of pesticides used in Prince Edward Island. A.M. Dunn. (PL) 136

An assessment of the effectiveness of 10 meter buffer zones in minimizing pesticide runoff from potato fields in Prince Edward Island. A. Denning, W.R. Ernst, M. Bernier, A. Cook, K.G. Doe, P.M. Jackman and G. Julien. (PL) 136

Assessment endpoints for ecological risk assessments of pesticides: a scientific perspective. G. Kaminski, P. Delorme, C. Kriz, V. Hodge, H. Mulye, R. Sebastien, C. Hart, P. Takacs, D. François and T. MacQuarrie. (PO) 137

"Fishy" business (aquaculture)/Historie d'eaux sales (aquaculture)

Chemicals in finfish aquaculture in Canada: a review of current practices and possible environmental effects. L.E. Burridge. (PL) 137

Impacts of freshwater aquaculture on the Canadian environment: towards science-based tools for sustainable management. S.L. Walker, S.S Dixit, D. Anderson, P.Y. Caux, P.A. Chambers, M.C. Chambers, L.A. Howes and L. Kingsley. (PL) 138

Monitoring of therapeutants and phycotoxins in kelp (*Laminaria saccharina*) and mussel (*Mytilus edulis*) cultured in proximity to salmon in an integrated system. D.H. Sephton, K. Haya, J.L. Martin, G. Boyer and T. Chopin. (PL) 138

Evaluation of the flesh quality of market-size farmed and wild British Columbia salmon. M.G. Ikonomou, D. Higgs, R. Devlin, B. Sukura, J. Oakes, S. McKinley, S. Jones, C. Dubetz, S. Balfry, J. Smith and N. Rowshandeli. (PL) 139

Toxic effects of jellyfish envenomation on fish in the northeast Atlantic. B.D. Johnston, H. Irvine and P.R. Boyle. (PL) 139

Potential endocrine disruption and mussel leukemia from finfish aquaculture. M.B. Engel, W.R. Ernst, S.D. St. Jean, F. Gagné and B.K. Burnison. (PL) 140

The effects of Salmosan® (Azamethiphos) on larval sea urchins and larval and juvenile scallops. L.E. Burridge, K. Haya and S.M.C. Robinson. (PL) 140

Persistent environmental contaminants measured in the sediments near salmon aquaculture sites. M.G. Ikonomou, J. Hellou, K. Hay and P. Sather. (PL) 140

Antibiotic resistant aerobic bacteria and oxytetracycline in sediments collected near salmon aquaculture sites in the Fundy Isles region, New Brunswick, Canada. K. Haya, B.T. Hargrave, K.G. MacKiegan, L.I. Doucette, S.M. Armstrong and F.S. Friars. (PL) 141

Best student paper awards/Prix pour les meilleurs exposés par des étuidants 142

List of authors/Liste des auteurs 143

List of participants/Liste des participants 146

Workshop proceedings/Compe rendus d'atelier 157

Plenary / Plénière

Bridge over troubled water: water issues over the past 30 Years / Bâtir des ponts eneaux troubles: trente ans de questions environnementales

Session Chair/Président: G. Toner

Linking aquatic science and policy. K. Brown. Environment Canada, Environmental Conservation Branch, Gatineau, QC.
In this presentation, two case studies are presented to demonstrate the linkages between science and policy at the different stages in the life cycle of an issue. Two issues are provided to illustrate the use of science to influence policy. The first example is acid rain, a mature issue that has completed the three stages of issue identification, policy development and implementation, and evaluation. The policies that were developed in the 1980's were based on critical loads for wet sulphate, which were driven by aquatic and atmospheric sciences. The second example is Canada's 1992 pulp and paper regulatory framework. The regulatory framework that was developed was strongly influence by research conducted in Canada which suggested that adsorbable organic compounds (AOX) was not the appropriate compound to regulate the toxicity of pulp and paper mills effluent. Not regulating AOX resulted in substantial cost savings and protected markets for the Canadian pulp and paper industry. These two examples clearly indicate why aquatic science and research need to continue to influence policy. The challenges that face scientists and risk managers in ensuring this continues will also be discussed.

Recent advances in freshwater research. D.R.S. Lean. Department of Biology, University of Ottawa, Ottawa, ON.
Research activities are traced from the burst of activity following the announcement that Lake Erie was dying in the 1960s. We witnessed the development of international leadership in Great Lakes Research in Canada and USA. We were proud of the awards and global leadership shown by researchers from the Experimental Lakes Area of Northwestern Ontario. Both provincial and federal government departments were the keepers of our waters while University based researchers grew from their short-term independent studies to integrated research teams often linked to national programs and priorities. We recognized advances as researchers became quantitative rather than qualitative. There is now less emphasis at our universities on basic courses in taxonomy (at any trophic level) yet it is changes in biodiversity where the effects of toxic chemicals are most often seen. While complementary research in environmental toxicology has been expanding, skills on basic limnological processes have been fading. The great promise of programs such as the Environmental Monitoring and Assessment Network (EMAN) seem to have lost momentum. The advice provided by the wise men of science has been silenced by the spin doctors working in public relations and advertising groups. We must ask if we are ready to meet the challenge of the next environmental problem.

Fifty ways to clean your river: the pulp and paper industry in the St. Lawrence River. J.-P. De Serres[1], G. Martin[1] and A. Lo[2]. [1]Domtar Inc., Montréal, QC; and [2]Domtar Inc., Cornwall, ON.

Abstract

The Canadian pulp and paper industry, with over one million employees and a net contribution of over $34 billion to Canada's trade balance, is without a doubt one of the most important industrial sectors in this country. It is, as well, one of the most heavily regulated industrial sectors. To comply with federal and provincial effluent regulations promulgated in the mid-1990's, all facilities in the Pulp and Paper Industry combined spent over $6.5 billion in the last decade. This has resulted in dramatic reductions in several parameters including toxicity, biological oxygen demand (BOD5), total suspended solids (TSS) and adsorbable organic compounds (AOX) discharges to receiving waters, along with the virtual elimination of dioxins and furans from mill effluents.

Nearly 60 mills in Ontario and Quebec discharge their effluents directly or indirectly into the St. Lawrence River. With Canadian mill effluent flows averaging up to 10% of receiving river flow, the improvement of water quality in St. Lawrence River rests largely on the pulp and paper industry's efforts. This paper deals with the background data defining the pulp and paper industry, the inception and promulgation of federal and provincial effluent regulations for the Sector as well as the Industry's success story in implementing various measures to comply with these regulations.

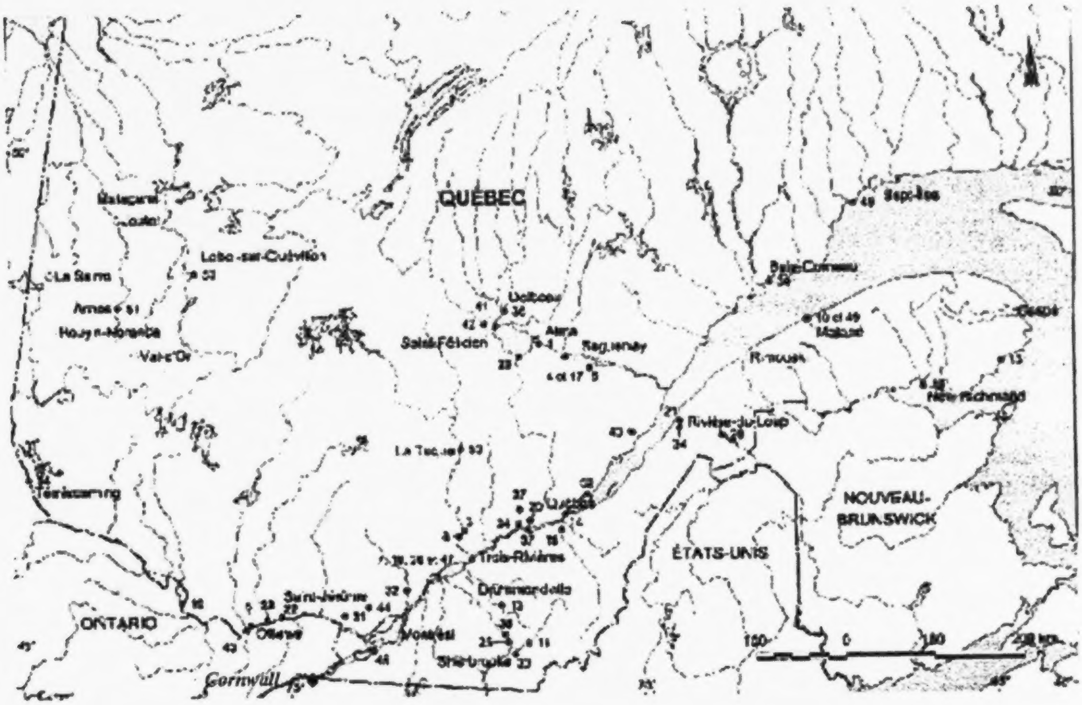
Introduction

In this day of increased environmental concern and public awareness, it has become important for industries to regulate and minimize their impact on the environment. This trend has become apparent in the Pulp and Paper Industry under the guise of increased governmental regulations and increased capital spending on environmental matters. Many questions come to mind when thinking about what has just been said: (i) how much money is the industry spending, (ii) what is it spending it on, (iii) what exactly is the government doing to regulate the industry, and finally (iv) is it working? To find answers to these questions we must look at the technical and economical factors that drive the pulp and paper industry and the intimate relationship it holds with the environment. What follows gives a current description of the pulp and paper industry from an economic point of view followed by the Domtar example, a description of certain laws and regulations affecting the industry, investments into environmental concerns along with the most important process modifications and the reasons for their implementation and the Industry's environmental performance.

The Canadian forest industry earned $1.9 billion in 2001, a 62% decrease over 2000 earnings of $5.0 billion. Sales revenue for 2001, at $54.6 billion, were $4.1 billion or 7% below 2000's record sales of $58.7 billion. The return on capital employed for the Canadian forest industry was 6% in 2001, down from 10.7% in 2000 and 9.0% in 1999, and consistent with the ten-year average.

The Canadian pulp and paper industry, when all forestry operations are included, is the largest industrial employer in Canada. The Canadian forest industry employed 246,000 full-time equivalent direct jobs in 2001, at an average annual compensation of $68,000 (including benefits). This represents a decrease of 5% or 11,500 jobs from 2000. The reduction of jobs was due primarily to weaker market conditions resulting in production curtailments. A further 738,000 full-time equivalents indirect jobs in other sectors of the Canadian economy can also be attributed to the forest industry, thus resulting in 984,000 total jobs for our Sector (PWC 2002). As a measure of the importance of the pulp and paper industry in the province of Quebec to the aquatic environment, there were 62 mills operating in 2001. These mills required the use of approximately 600 million cubic meters of water in 2001 alone, with the generated wastewaters being discharged directly into the St. Lawrence River or by one of its many tributaries (Fig. 1, MENV 2001a).

With 2002 sales of close to $5.5 billion, Domtar is the third largest producer of uncoated free sheet paper in North America. It is also a leading manufacturer of business papers, commercial printing and publication papers, and technical and specialty papers. Domtar manages close to 22 million acres of forestland in Canada and the United States, and produces lumber and other wood products. Domtar has 12,000 employees across North America (Domtar Inc. 2002). The company also has a 50% investment interest in Norampac Inc., the largest Canadian producer of containerboard. More details on Domtar can be found at www.domtar.com.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Sept-Îles
New Richmond
Saguenay
Témiscaming
Val-d'Or
Amos
La Sarre
Lebel-sur-Quévillon
La Tuque
Saint-Jérôme
Saint-Félicien
Matane
Gaspé
100
0
100
200 km

Fig. 1. Location of pulp and paper mills in Quebec (with Cornwall, ON, highlighted)

Laws and regulations

As of May 7th 1992, all Canadian mills became subject to the *Pulp and Paper Effluent Regulation*s, under the *Fisheries Act*. The regulations were created to set rules as to how much Biochemical oxygen demand (BOD5), total suspended solids (TSS) and acutely lethal effluent could be discharged into Canadian rivers (Table 1). Furthermore, the regulations obliged Canadian pulp mills to follow the environmental effects monitoring (EEM) program. The EEM program is used to assess the adequacy of national regulations for protecting fish and their habitat along with the use of fish resources.

On a provincial level, Quebec mills are not limited in the amount of BOD5 and TSS in their final effluent if they discharge into municipal sewage systems (which is the case for 11 mills). If the mill discharges directly into a river, and depending if it was built before or after October 22nd 1992, it must meet the discharge limits (Table 2). In addition, Ontario kraft and mechanical mills must abide by the following regulations pertaining to their final mill effluent discharges (Table 3).

In the past decade, Canadian mills have made significant modifications to their pulping and papermaking processes as a way to render their effluent discharges compatible with the aforementioned new effluent regulations. The elimination of dioxins and furans from mill effluent has been the primary concern driving the pulp and paper industry towards cleaner effluent, although tighter governmental regulations for BOD5, TSS and acute toxicity adopted in 1992 were also motivating factors. Having spent over 6.5 billion dollars in the last 10 years (Ekono/Douplan 1996).

Table 1. Effluent limits – Federal

Parameters		Units	Maximum Limit
TSS	Daily	kg/adt	12.5
	Monthly	kg/adt	7.5
BOD$_5$	Daily	kg/adt	18.75
	Monthly	kg/adt	11.25
Dioxin – 2378 – TCDD		pg/L	15
Furan – 2378 – TCDF		pg/L	50
Acute fish toxicity		LC$_{50}$	≥ 100%

Table 2: Effluent limits in Quebec

Parameters		Units	Maximum Limit	
			Existing	New
TSS	Daily	kg/adt	16.0	6.0
	Monthly	kg/adt	8.0	3.0
BOD$_5$	Daily	kg/adt	8.0	4.0
	Monthly	kg/adt	5.0	2.5
AOX	Daily	kg/adt	1.0	0.3
	Monthly	kg/adt	0.8	0.25
PCB		µg/L	3.0	3.0
Dioxins and furans		pg/L	15	15
Hydrocarbons		mg/L	2.0	2.0
Acute fish toxicity		aTU	1.0	1.0
pH			6 – 9.5	6 – 9.5
Temperature		°C	<65	<65

Table 3. Bleached and unbleached kraft mill effluent limits in Ontario

Parameters	Units	Mills			
		Bleached		Unbleached	
		Daily	Monthly	Daily	Monthly
TSS	kg/adt	13.4	7.9	7.8	4.6
BOD$_5$	kg/adt	10.0	5.0	5.8	2.9
AOX	kg/adtbp	1.03	0.8	N/A	N/A
Total phosphorus	g/adt	280	170	83	50
Chloroform	g/adt	3.72	1.88	2.17	1.09
Dioxins/furans/TEQ	pg/L	60		N/A	
Dioxin – 2378 – TCDD	pg/L	20		N/A	
Furan – 2378 – TCDF	pg/L	50		N/A	
Toluene	g/adt	0.22		0.28	0.22
Phenol	g/adt	0.41		0.24	
Acute fish/Daphnia toxicity	LC$_{50}$	≥ 100%			
pH		6 – 9.5			

on modifying physical, chemical and biological processes, the industry has strived to increase its recycling capacity, reduce its overall emissions and improve environmental performance all the while increasing production (Martel et al. 2000, Kovacs et al. 2002).

Elimination of chlorinated organics from final mill effluent has pushed the industry to find the source of these compounds and eliminate their toxic effects on fish and their habitat. Consequently, many process modifications were implemented when the industry adopted such techniques as: chlorine dioxide substitution, elemental chlorine free (ECF) and totally chlorine free (TCF) processes, increased delignification prior to bleaching, minimizing black liquor losses and carryover to the bleach plant, better condensate handling, better brown stock washing, increased white liquor recovery, process water recycling, colour removal and optimizing secondary treatments through pH control and increased aeration capacity (Martel et al. 2000, Kovacs et al. 2002). Some of these modifications deserve a closer look and are discussed below.

ECF processes were first introduced because ClO2 was found to be very efficient at preserving pulp strength while still having good bleaching characteristics, allowing production of stronger paper at a lower cost (for no more than 25-30% substitution). In the 1990's, the industry moved to higher substitution (55-100%), despite the increased production cost, in order to comply with emerging regulations that required mills to minimize the amount of chlorinated organics created in the bleaching process. Studies have shown that chlorinated compounds, present in the effluent from mills using ECF bleaching processes, have a much lower chlorine substitution than those produced with elemental chlorine bleaching, which generally means that they are less persistent and bioaccumulative (Fig. 2, Bright et al. 1998).

In 2000, ECF bleaching was used to produce 10.7 million tons of bleached chemical pulp (BCP), which represented more than 90% of the Canadian market for BCP (Allian. Environ. Technol. 2000). For economic reasons, TCF bleaching has not become as popular as ECF because it was found to be more costly, difficult to stabilize and poorly efficient. Furthermore, regardless of the fact that no chlorine is used in the bleaching plant, there are still wood extractives that seem to cause biochemical responses and changes in fish reproduction parameters present in the mill effluent. These non-chlorinated compounds keep TCF bleaching from being more environmentally compatible than other bleaching techniques that produce chlorinated compounds.

Secondary treatment

This process by which microorganisms, namely fungi, bacteria, rotifers and algae, are put in contact with the mill effluent and feed on the available organic matter thereby drastically reducing the amount of organic pollutants present at the final mill effluent. The types of secondary treatment processes differ by the means with which they allow contact between the mill effluent and the microorganisms. A residence time of a few hours to many days can be necessary, depending on the type of treatment used, in which time oxygen supply, temperature and pH must be closely monitored to avoid sudden and lethal changes to the bacterial living environment (NRCan. 1994a). The secondary treatment plants most commonly used in Canada are activated sludge, which include sequential batch reactors, aerated stabilization basin and biofilters.

Secondary treatment has become commonplace for modern mills because, when properly optimized, they biologically degrade BOD5, phenolic compounds and resin acids to proportions of 70% to 95%, which usually eliminates acute toxicity (MENV 2001b). As well, secondary treatment was also found to diminish and sometimes completely eliminate sub-lethal toxicity in fathead minnows and *Ceriodaphnia* along with reducing "MFO-inducing potency" from bleached Kraft, thermomechanical and chemi-thermomechanical pulp mill effluents.

At times, a portion of the investments made to install wastewater treatment plants can be recovered

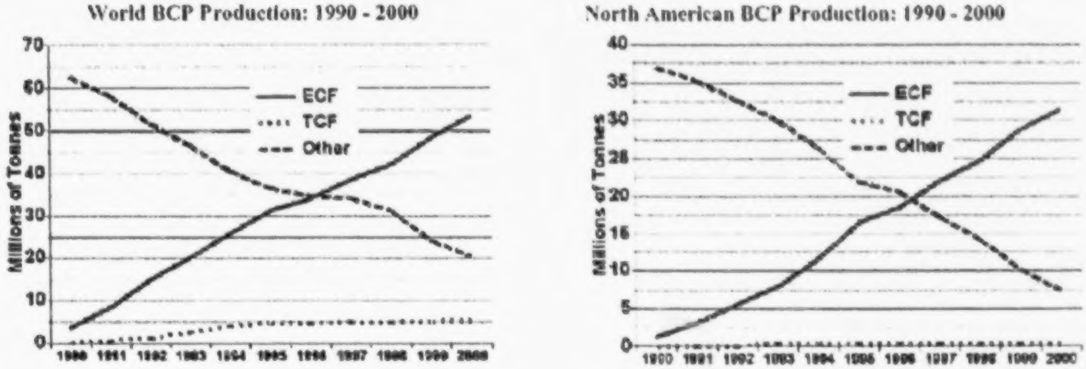

Fig. 2. World bleached pulp production (Bright et al. 1998)

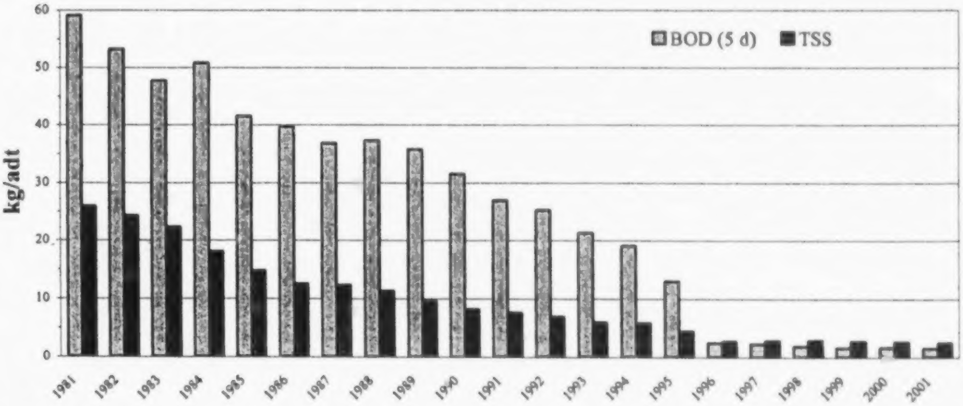

Fig. 3. BOD_5 and TSS discharges in kg/adt since 1981

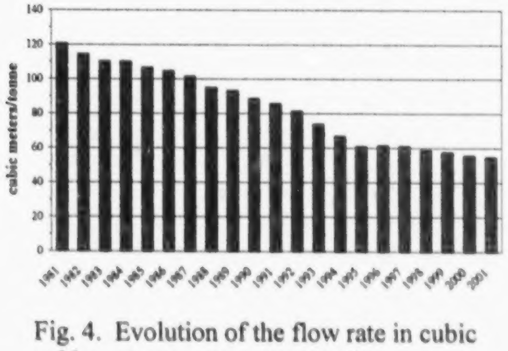

Fig. 4. Evolution of the flow rate in cubic cubic meters per ton since 1981

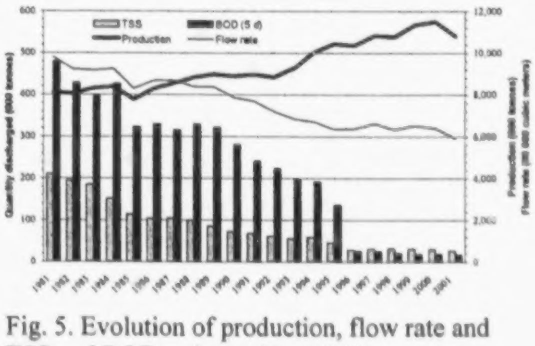

Fig. 5. Evolution of production, flow rate and TSS and BOD_5 rejects since 1981

by reducing the mills' water consumption, hence diminishing energy costs and capital expenditures. A good water use reduction program also enables mill expansions without overloading of the treatment plant, reduces costs of moving and aerating effluent and also lowers treatment costs for incoming fresh water (NRCan 1994b).

The evolution of BOD5 discharged in receiving waters and the pronounced drop in BOD loadings reported in the mid 1990s (Fig. 3). Secondary treatments were also very effective at eliminating acute toxicity, while chlorinated organics such as dioxins and furans, whose concentrations became non-detectable in mill effluent across Quebec as of 2000, were virtually eliminated once bleaching processes were modified (MENV 2001b).

Domtar, as part of its environmental policy, has committed to conduct business in a manner that protects the environment, conserves resources and ensures sustainable development. Domtar seeks continuous improvement in its environmental performance by setting, reviewing and updating our environmental objectives. Domtar's environmental policy can be found at http://domtar.com//media/En_EnviroPolicy.pdf. Cornwall, as part of Domtar, must adhere to all the points that make up Domtar's environmental policy. To that effect, the mill has put in place several operating measures to ensure that the Policy is implemented and respected, including the certification of its operations and management systems under the ISO 9002 and ISO 14001 systems.

Environmental performance (1992-2002)

Biochemical oxygen demand is used in wastewater treatment plants as a way to evaluate the quantity of organic material in the mill effluent. Higher values of BOD5 (usually evaluated over 5 d) indicate higher concentrations of organic material in the effluent, which is undesirable. Indeed, high BOD5 values in receiving waters cause reductions in dissolved oxygen concentrations, which can have serious effects on fish and benthos development in these rivers. Another way to measure the impact of wastewater on receiving waters is by measuring the amount of total suspended solids (TSS) in the river or treatment plant for monitoring reasons. Suspended solids are closely related to the amount of microorganisms present in a river, they also diminish the amount of light penetrating into the water. Studies have shown that large amounts of suspended solids in rivers can, even though they are made of non-toxic material, impinge the growth of certain algae by depositing themselves on riverbeds and also cause deterioration in fish metabolism by causing serious irritation to gill tissue over an extended period of time (Smook 1994). Furthermore, aerobic bacteria are killed from accumulation of oxygen depleting organic material on riverbeds, which facilitates anaerobic bacterial growth causing foul smelling methane and hydrogen sulphide emanations (Smook 1994).

As seen in Fig. 3, the data for the 62 mills operating in the province of Quebec in 2001 show that the quantities of BOD5 and TSS discharged from 1981 to 2001 have diminished drastically (MENV 2001b). This 97% decrease per ton of production in BOD5 discharged is also accompanied by an 89% decrease in TSS even when the industry showed a production increase of 40% over the same period. These numbers reflect the increased use of secondary wastewater treatment plants and a number of process changes (see Sections 4.2 and 4.3) that have become necessary for Quebec mills in order to comply with provincial and national effluent discharge laws.

Water reduction projects were first put into motion in order to diminish energy losses and increase productivity. Mills have taken a variety of approaches to reduce their water consumption: some reuse the process/cooling water, optimize their cooling water usage or improve the process control and modernize their equipment (see Fig. 4, MENV 2001a, Kovacs et al. 2003). Even though it is environmentally sound to do so, reducing the amount of effluent generated can cause build-up type problems such as suspended solids buildup, increase in dissolved solids and thermal buildup by simply recycling water used for transporting solids, washing and chemical recovery. An increase in corrosion, foaming, foul odors, pitch, dirt, wire and felt plugging. These all good reasons why the industry is reticent about effluent reduction, but upcoming energy costs may make it necessary if mills want to stay competitive and efficient.

In addition, since lethal toxicity regulations are concentration-based, it is only prudent to ask if reducing water consumption in a mill would not concentrate the final effluent, thus concentrating the toxic elements contained within and augmenting toxicity measurements. A study involving 73 Canadian mills studied the effect of flow rate reduction in pulping mills and effluent toxicity using mainly rainbow trout and *Daphnia* toxicity tests (Kovacs et al. 2003). It was found that mills discharging their cooling waters directly into receiving waters were more problematic to the environment than other mills by exceeding federal or provincial effluent regulations more often. But, no clear trend was detected between frequency of toxicity episodes and water usage, indicating that toxicity and effluent reduction may or may not be related. It is also noteworthy that, although water consumption has gone down in pulp mills, productivity has nevertheless increased (see Fig. 5, MENV 2001a).

AOX formation in Kraft and sulphite mills generally stems from the cooking and bleaching sections of the chemical process intended to delignify woodchips and whiten the subsequent pulp. Spent cooking liquor carried over to the bleach plant, excess filtrates from the bleach plant sent to the effluent treatment plant and certain defoaming agents are the main contributors to AOX presence at the outfall of pulp mills (Carey et al. 202). The non-fibrous organic compounds carried into the bleaching plant are normally treated with dioxide in order to separate them from the desirable fibrous organic compounds (e.g., cellulose, hemicellulose), thus creating adsorbable organic halides (chlorinated organic compounds adsorbable by activated carbon). Even though over 300 AOX compounds are produced from the exposure of organic compounds to halogens (BC Min. Wat. Land, Air Prot. 2002), many have been classified into one of the following families: dioxins, furans, chlorophenols and chlorinated fatty and resin acids (Carey et al. 2002). These five families are known to have toxic, bio-accumulative and carcinogenic properties in crabs, clams and fish. Significant effort has been put into characterizing the effects of two of these families, particularly dioxins and furans. It was found that 2378-TCDD and 2378-TCDF were exceptionally toxic and persistent in aquatic life, affecting fish and benthic communities alike. Canadian laws established in 1992 have set the limits for dioxins and furans to "non-detectable" (Canada Gazette 1992), also prohibiting any discharge of effluent that is acutely lethal to fish (see Table 1). Despite a steady increase in bleached pulp production, dioxin and furan levels in pulp mill effluents have been virtually eliminated (Fig. 6).

Acute toxicity is generally established with either *Daphnia* (fresh water fleas) or rainbow trout. The use of these tests is apparent when trying to evaluate if wastewater treatment plant effluent are lethal to river dwelling organisms. *Daphnia magna* or *Daphnia pulex*, depending on the hardness of the water tested, were chosen for routine toxicity tests for a number of reasons (Environment Canada 1990a). *Daphnia* are present in large amounts of Canadian freshwater bodies and throughout a wide range of habitats. They are a significant food source for juvenile salmonids and represent an important link in the aquatic food chain. Their small size and relatively short life span make these organisms easy to culture, sample and transport. Finally they are sensitive to a broad range of aquatic contaminants.

Median lethal concentrations, time to 50% mortality and median effective concentrations (LC50, LT50 and EC50 respectively) are calculated in order to characterize the toxicity of the solution. As mentioned earlier, rainbow trout is also widely used in acute toxicity testing. In the 1970's Environment Canada published a series of regulations and guidelines for acute lethality testing with rainbow trout (EPS: 1971, 1974, 1977a-c) (Environment Canada 1990b). For two decades, the Canadian Government has used fresh water tests with rainbow trout (*Oncorhynchus mykiss*)

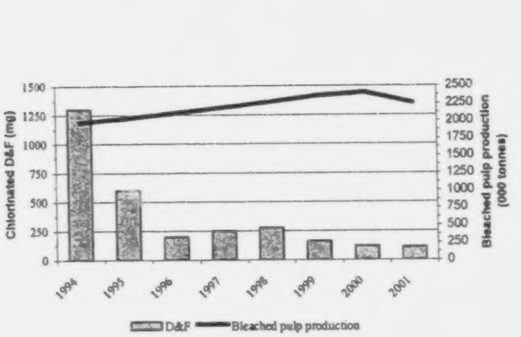

Fig. 6. Dioxin and furan discharge evolution
In parallel with production since 1994

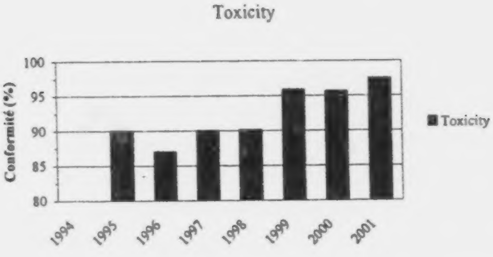

Fig. 7. Compliance of Quebec mills
to toxicity regulations since 1994

extensively and routinely, thus creating an important toxicological data bank for this species. LC50 and LT50 are normally calculated over a 96 h period in order to characterize the toxicity of a solution. In summary, "the overall performance of mills in consistently meeting the regulatory effluent toxicity limit in tests with rainbow trout was approximately 90% in 2000. This represents a substantial improvement since 1995-96, with some levelling off since 1997 (Kovacs et al. 2003)."

Conclusions

The pulp and paper industry used approximately 600 million cubic meters of water in the province of Quebec in 2001 alone, and being the largest industrial employer in Canada, when including all forestry operations, is naturally attributed a large amount of responsibility towards the state of the environment. As such, it has spent over 6.5 billion dollars in the last ten years in process modifications in order to increase recycling and reduce overall emissions and water usage. As an example of environmental leadership, Domtar has spent over 380 million dollars in the last ten years to comply with environmental regulations all the while increasing its production and diminishing its overall water usage. Most of the reductions measured in environmental indicators such as BOD5, TSS and AOX can be attributed to the installation of secondary treatment systems in a vast majority of Canadian mills. In fact, secondary treatment systems have become commonplace for modern mills because, when properly optimized, they biologically degrade BOD, phenolic compounds and resin acids to proportions of 70% to 95%. When considering the results obtained to date, there are no embellishments made when stating that the condition of St. Lawrence tributaries, and thus the St. Lawrence River itself, are improving and will continue to improve with time, thanks in part to the efforts made, over the last ten years, by the Pulp and Paper Industry.

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A new approach to water management. R. Findlay. Pollution Probe, Water Programme, Ottawa, ON.

Pollution Probe sees a new approach to water management that takes a positive, progressive, prevention-oriented and forward looking view of what is needed to achieve future sustainability. This new approach is based on a broad retrospective analysis of how we got here, and the key turning points and milestones that marked the path. While the new approach recognizes that many positive gains have been achieved over the last few decades, it suggests that new approaches, new partnerships and new institutions are needed to ensure protection of our water resources for future generations.

Safeguarding Canada's freshwater resources: international aspects. A.L. Hamilton. Consultant (previously for the Commission for Environmental Cooperation), Ottawa, ON.

Canada has increasingly recognized the need for international cooperation in conserving and protecting aquatic resources. The *Boundary Waters Treaty* of 1909 and the International Joint Commission that it established have provided much of the institutional framework for preventing and resolving conflict over boundary and transboundary aquatic ecosystems. The initial focus on irrigation and power generation has evolved although water quantity continues to be important. Water quality concerns, once primarily related to water borne diseases, were initially allayed through the chlorinating of drinking water. Nutrient pollution (eutrophication), especially in the Great Lakes, eventually became so severe and so obvious that it could no longer be ignored. The resulting *Great Lakes Water Quality Agreement* of 1972 provided the initial framework and the 1978 Agreement added a major focus on persistent toxic substances and recognized the need for an ecosystem approach. The initial emphasis on local point sources of industrial and municipal pollution shifted and broadened to include the more difficult non-point sources, especially those resulting from agricultural activities and the long-range atmospheric transport of contaminants. Air borne acid rain precursors, mercury and persistent organic pollutants are now recognized as major national and international threats to regions remote from sources of pollution. Acidification of poorly buffered lakes and the bioaccumulation of persistent toxic in aquatic food chains, especially in high latitude and high altitude aquatic ecosystems are major international issues now being addressed through international agreements and action.

Contributed Papers/Documents contribués

Building a stronger bridge between laboratory and field / Bâtir un pont solide entre le laboratoire et le terrain

Session Co-chairs/Présidents: G. Gilron and/et D. Farara

Algae, cyanobacteria and aquatic macrophytes for ecotoxicity tests: maintenance and sources of axenic cultures. J.C. Acreman. University of Toronto Culture Collection (UTCC), University of Toronto, Toronto, ON.

Axenic cultures of algae, cyanobacteria and aquatic macrophytes used in ecotoxicity testing require specialized care to ensure continued viability, purity and over long periods. Rigid standards in sterile technique are necessary to continually produce axenic stock cultures of these organisms during the frequent manipulations required in serial transfer. The use of a well-maintained laminar flow hood is essential for all manipulations of axenic stock cultures. The hood should be sterilized before use by an ultraviolet lamp and by washing the work area with a disinfectant such as 70% ethanol or bleach diluted 1:100. A good basic rule to follow is to treat open culture vessels as carefully as you would an open wound. Use only sterile instruments and media; flame openings of glass culture vessels to destroy contaminants. Hands should be washed with antibacterial soap followed by periodic cleansing with waterless hand cleaner such as One-Step while working in the hood.

Multiple cultures of each strain should be made at each transfer cycle to ensure that at least some of the cultures are retained as axenic cultures. Replicate cultures should be kept in 2 locations. Some hardy species such as *Pseudokirchneriella subcapitata* (= *Selenastrum capricornutum*) and *Chlorella vulgaris* can be refrigerated for several weeks to prolong viability. In order to ensure optimal viability, preservation of the strains by at least two methods is the ideal, to avoid the strains being lost or contaminated. An alternate method to the serial transfer technique is cryopreservation in liquid nitrogen. This technique minimizes the potential for loss and contamination of cultures in long-term preservation and is practical when large numbers of cultures are involved.

Axenic cultures of algae, cyanobacteria and aquatic vascular macrophytes can be maintained for long periods using serial transfer. At the University of Toronto Culture Collection, species such as: *Anabaena flos-aquae*, *Dunaliella tertiolecta*, *Navicula pelliculosa*, *Phaeodactylum tricornutum* and *Pseudokirchneriella subcapitata* are maintained at 20°C in defined media. For freshwater species the media generally used are: BG-11 for cyanobacteria, CHU-10 for diatoms and BBM for green algae; artificial sea water medium, ESAW, is used for marine species (formulations are found on the UTCC website www.botany.utoronto.ca/utcc). The aquatic vascular macrophytes used in ecotoxicity testing, *Lemna minor*, *Lemna gibba*, *Myriophyllum sibiricum* and *Myriophyllum spicatum* are maintained in UTCC at 25°C in Hoagland's E+ and Andrew's media respectively. Methods for testing sterility of axenic cultures include subculturing to liquid or solid media containing high levels of organic substances and examination using phase contrast or fluorescence microscopy.

Purification of contaminated cultures can be performed but it is difficult, time-consuming and unpredictable. Methods for purification include mechanical re-isolation and application of antibiotics, or in the case of *Lemna*, a solution of sodium hypochlorite (bleach) in a 10% solution can be used. For further information on purification see the References section of the UTCC website. Due to time factors and uncertain results in purification, the best option is usually to replace the culture.

As the quality of the cultures used in ecotoxicity tests is critical to the results, it is important to obtain authentic and axenic cultures from a reliable source. In North America, cultures for ecotoxicity testing are available from: (i) University of Toronto Culture Collection (UTCC), Botany Department, University of Toronto, Toronto, ON, supplies freshwater and a few marine strains of microalgae, cyanobacteria and aquatic vascular macrophytes. Further details are below; (ii) Provasoli-Guillard Culture Collection of Marine Phytoplankton (CCMP), West Boothbay Harbor, ME, supplies marine microalgae; (iii) University of Texas Culture Collection (UTEX), University of Texas at Austin, TX, supplies freshwater microalgae and some marine species; and (iv) The American Type Culture Collection (ATCC), Manassas, VA, supplies a limited selection of freshwater species of algae and cyanobacteria in frozen condition.

In addition to serving as a centre for the supply of axenic cultures for the Environmental Effects Monitoring (EEM) program, the UTCC also functions as a fee-based, phycological resource centre. Some of services provided include training sessions in methods of culturing and purifying algae, cyanobacteria and aquatic macrophytes, supply of sterile media, and custom-isolation of algae and cyanobacteria. Funds for the basic maintenance of the UTCC are provided by a Major Facilities Access grant from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Can benthic invertebrate communities directly affect laboratory toxicity test organisms in sediment quality studies? M.D. Paine[1], E. DeBlois[2], S.A. Whiteway[2], U. Williams[3] and F. Power[3]. [1]Paine, Ledge & Associates , North Vancouver, BC; [2]Jacques Whitford Environment Ltd., St. John's, NL; and [3]Petro-Canada, St.John's, NF.

Laboratory toxicity tests and monitoring of *in situ* benthic invertebrate communities are frequently used in sediment quality studies. These tools are considered independent laboratory (toxicity tests) and field (invertebrate communities) measures of biological responses to physical or chemical alteration of sediments. Evidence for effects is more convincing when both measures respond negatively to alterations. We present a case study where laboratory and field biological effect measures do not appear to be independent. Luminescent bacteria (Microtox®) responded negatively to sediment samples from 10-20 of 50 stations. Those negative laboratory responses were unrelated to project alterations from offshore oil drilling, and were associated with high invertebrate richness and diversity. The negative laboratory responses were strongly correlated with sediment total inorganic carbon (TIC) concentrations, a potential indicator of shell or exoskeleton fragments. Our working hypotheses are that some by-product of decomposition of larger invertebrates negatively affects the bacteria, and that decomposition will be greater where diversity and abundance of those larger invertebrates is greater. If you are sessile, you die where you live. When these results were previously presented, the more general and intriguing hypothesis that sessile invertebrates emit chemical secretions (i.e., indulge in chemical warfare) that may affect laboratory toxicity test organisms was raised.

Toxicity and bioaccumulation of tributyltin (TBT) from freshwater harbour sediments. A.J. Bartlett[1], D.G. Dixon[1], R.J. Maguire[2], S.P. Batchelor[2] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

The toxicity of tributyltin (TBT) has been well documented in marine organisms, however few data are available describing the impact of TBT in freshwater environments. Previous research in our laboratory has investigated the effects of TBT on freshwater invertebrates using spiked-sediment toxicity tests. This study was designed to evaluate the ability of these laboratory tests to predict toxicity and bioaccumulation of TBT in the environment. Sediments were collected from five locations that have been historically contaminated with TBT: Montreal, Kingston, Toronto, Hamilton, and Port Weller. Four-week toxicity tests measuring survival, growth, and bioaccumulation were conducted using 0-1 week old *Hyalella azteca* (a freshwater amphipod). In the event that bioaccumulation could not be measured due to high mortality, two-week bioaccumulation tests were conducted using 4-6 week old *Hyalella*. There was no effect of TBT on survival or growth of *Hyalella*. There was a linear relationship between TBT in Hyalella and TBT in sediments (r2=0.72), although TBT in field sediments was bound more tightly than in spiked-sediments. Bioaccumulation of TBT in *Hyalella* exceeded levels expected to cause reproductive effects in some sediments from Kingston, Montreal, and Port Weller. This research suggests that environmental concentrations of TBT in harbour sediments are still capable of causing chronic toxicity in freshwater invertebrate populations.

Using tissue residue effects as an ecotoxicological framework to establish links between laboratory and field studies. M.H. Salazar. Applied Biomonitoring, Kirkland, WA.
Bioaccumulation is the link between environment and organism and is proposed as a link between laboratory and field studies. Links for characterizing exposure are established by combining measurements of external exposure (water and sediment chemistry) with the dose (tissue chemistry). Links for characterizing effects are established by combining the dose (tissue chemistry) with a response (single species bioassay and community endpoints). These bioassay and community endpoints can be linked by combining those measured in the lab and in the field. Integrating the tissue residue effects paradigm, caged bivalves, and ecological risk assessment has several advantages over more commonly used approaches in ecotoxicology. This weight-of-evidence approach provides (i) an estimate of chemical potency and chemical effects in the same organism and at the same time, (ii) combined measures of exposure and effects over space and time, and (iii) controlled field experiments under environmentally realistic and site-specific conditions. *In situ* studies with caged bivalves facilitate collecting synoptic tissue residue and effects data for ecotoxicological assessments and ecological risk assessment. To demonstrate the utility of this approach, copper will be used as a case study for quantifying critical body residues associated with adverse effects in bivalves. Paired critical body residues and effects will be compared in freshwater and marine bivalves. Survival, growth and reproductive effects will be emphasized because these endpoints are most commonly measured endpoints.

De-mystifying mixtures / La démystification des mélanges

Session Co-chairs/Présidents: U. Borgmann and/et D.G. Dixon

Modelling multiple metal binding to fish gills and to natural organic matter. R.C. Playle, S. Smith and A. Winter. Department of Biology, Wilfrid Laurier University, Waterloo, ON.
Metal-gill binding models and biotic ligand models in general are designed to predict metal toxicity to aquatic organisms using site-specific water chemistry (see special BLM issue in Comp. Biochem. Physiol. 2002, **133**C, 1,2). Cation competition at metal binding sites on fish gills and anionic complexation in the water both reduce metal binding to the gill membrane, reducing metal toxicity. But in mixtures, metal cations themselves will compete at the gills and will also compete at binding sites on natural organic matter (NOM). If metals bind to the same sites on fish gills, modelling using the toxic unit (TU) concept indicates greater than strict additivity at low metal concentrations (at 1 TU), due to the non-linear nature of the models. Competition for metal binding sites on NOM will be illustrated using changes in excitation-emission scans as metals bind to NOM; these scans are optical "fingerprints" of NOM from different sources. Metal binding is expected to be complex in metal mixture-gill-NOM systems because of the non-linear nature of the multiple interactions.

Concentration addition vs effects addition models: evaluation of 10-metal mixture toxicity. W.P. Norwood[1], D.G. Dixon[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.
Often at sites of contamination, several metals are elevated simultaneously and the potential for joint toxicity exists. However, historically no clear and consistent pattern of joint toxicity has emerged. Any given metal combination has resulted in impacts that range from antagonistic to less than additive, additive, more than additive, through to synergistic when the toxicity is expressed relative to the concentration of the metals in water or sediment. Body concentrations of single metals are often a more useful indicator of toxic effects than water or sediment concentrations. Therefore, toxic units, based on body concentrations as well as water concentrations, are compared in a concentration addition model to evaluate the chronic impact of 10 metal mixture (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl and Zn) exposures on *Hyalella azteca*. In addition, allometric models relating body concentrations to mortality and water concentrations to mortality, are compared in an effects addition model.

Synergistic toxicity of mixtures: metal/PAH/oxyPAHs impacts via catalytic generation of reactive oxygen species (ROS). B.M. Greenberg, X.D. Huang, T.S. Babu, M. Lampi, J. Nykamp and F. Xie. Department of Biology, University of Waterloo, Waterloo, ON.
PAHs and heavy metals are two prevalent classes of persistent contaminants in the environment. Because they often share the same production sources, they frequently co-exist in contaminated environments. However, most toxicological studies of these contaminants are carried out on individual chemicals. Few studies have focused on mixtures of the same class of contaminants, and even fewer investigate the hazards of mixtures of different classes of contaminants such as metals, PAHs and oxyPAHs. Therefore, the hazards of mixtures of these classes of chemicals have not been evaluated. Further, the mechanisms of the effects of these classes of chemicals are virtually unknown. We have found that mixtures of metals (e.g., Cu and Cd) and oxyPAHs (e.g., hydroxy anthroquinones and phenanthrene quinone) cause synergistic toxicity. The synergistic effects are induced by different modes of action of metals and oxyPAHs. For instance, 1,2 dihydroxyanthquinone inhibits cytochrome b/f and cytochrome c reductase, and thus, interrupts electron transport in chloroplasts and mitochondria, respectively. Cu++ uses a catalytic mechanism to generate ROS by accepting electrons from reduced quinines, and succinate or NADH dehydrogenase. Thus, photomodified PAHs inhibit electron transport, while metals harvest the electrons from the blocked bioenergetic membranes to generate high levels of ROS. This results in synergistic toxicity due to the high ROS loads. We have observed this type synergistic toxicity in plants, aquatic animals, bacteria and mammalian cells. This research reveals an important general mechanism of environmental toxicity.

Why does crude oil cause blue sac disease in larval fish? P.V. Hodson, C. Noble, H. Orr, P. Quereshi, S.P. Tabash and M. Bauder. Department of Biology, Queen's University, Kingston, ON.
Embryos and larvae of fish exposed to crude oil develop blue sac disease (BSD), a syndrome characterized by edema, haemorrhaging, deformities, and CYP1A induction. BSD was correlated to concentrations of C1-C4 alkylphenanthrenes (APs), but not to unsubstituted polynuclear aromatic hydrocarbons (PAH). The mechanism of toxicity of APs is related to their oxygenation by CYP1A enzymes to benzylic alcohols (alkyl group hydroxylation), and signs of toxicity suggest oxidative stress. We co-exposed larval trout to retene (7-isopropyl-1-methylphenanthrene; CYP1A inducer) and to compounds that induce or down-regulate the cyp1A gene, inhibit CYP1A activity, or compete for glucuronide conjugation. Treatments affecting cyp1A gene expression or that competed for glucuronide conjugation had little effect on retene metabolism or toxicity, suggesting that toxicity was not due to easily excreted metabolites. Inhibition of CYP1A activity reduced excretion rates of retene,

changed metabolite profiles in tissues and bile, and increased toxicity to larvae. Hence, oxygenation of alkyl PAH by CYP1A enzymes plays a key role in their metabolism and toxicity, and inhibition of oxygenation causes the retention of the toxic form, either parent retene or a specific metabolite. The toxicity of unsubstituted phenanthrene, which is not a CYP1A inducer, increased when metabolism and excretion were accelerated by co-exposures with an inducer, indicating that the toxicity of oil may not be easily understood by considering the components individually.

Toxicity of oil sands to early developmental stages of fathead minnows (*Pimephales promelas*). M.V. Colavecchia[1], S. Backus[2], P.V. Hodson[1] and J.L. Parrott[2]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

This study examines the effects of oil sands exposure on the early life stages (ELS) of fathead minnows (*Pimephales promelas*). Sediments within and outside natural oil sand deposits were collected from sites along the Athabasca River, Alberta, Canada. ELS toxicity tests were conducted on controls, natural river bitumen deposits, reference sediments and oil refining wastewater pond sediments. Eggs and larvae were exposed to 0.49-25 g sediment/L, and were checked daily for mortality, hatching, malformations, growth and CYP1A induction (using an immunohistochemical method). Natural bitumen and oil refining wastewater pond sediments caused significant exposure-related increases in ELS mortality, malformations, and reduced growth. Larval deformities included edemas, hemorrhages and spinal malformations. Exposure to reference sediments and controls showed negligible embryo mortality and malformations, and excellent larval survival. Sediment analyses using HRGC-MS revealed high concentrations of higher molecular weight and alkyl-substituted PAH in natural bitumen (TPAH concentrations 310-600 mg/kg) and oil mining pond sediments (3,500 mg/kg). These include: C2-C4 phenanthrene/anthracene, C1-C4 fluoranthene/pyrene, benz(*a*)anthracene/chrysene and C1-C2 benzofluoranthene/pyrene. ELS sediment toxicity tests may prove to be valuable tools in the monitoring and assessment of petroleum pollution.

Histopathological effects of naphthenic acids and salinity to goldfish (*Carassius auratus*). V. Nero[1], A.J. Farwell[1], A. Lister[2], L.E.J. Lee[3], G.J. Van Der Kraak[2] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Zoology, University of Guelph, Guelph, ON; and [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

The extraction of bitumen from the Athabasca oilsands deposit (Alberta, Canada) produces vast volumes of process-affected water containing naphthenic acids (NAs), polycyclic aromatic hydrocarbons (PAHs), and high salinity. Previous work has shown that experimental reclamation sites containing elevated levels of naphthenic acids and salinity induce severe histopathological alterations in goldfish (*Carassius auratus*). But, due to the many confounding chemical factors that exist in process-affected water, the precise nature of the factor that is responsible for the observed effects could not be determined. Therefore, the objective of this study was to examine the independent and interactive effects of naphthenic acids (extracted from process-affected water) and salinity to goldfish histopathology. The results of this study are compared to previous work to determine the causative agent of histopathological changes associated with exposure to high levels of oilsands process-affected water.

Comparative toxicity of water-accommodated fractions of oil and dispersed oil to marine fish larvae. C.M. Couillard[1], K. Lee[2], B. Legare[1] and S. St. Pierre[1]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mt. Joli, QC; and [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Applications of chemical dispersants may increase the risk of toxic effects to early life stages of fish by increasing their exposure to polycyclic aromatic hydrocarbon (PAH). Weathered Mesa light crude oil and filtered seawater with or without the addition of Corexit® 9500 were used to prepare water-accommodated fractions of dispersed crude oil (DCWAF) or crude oil (WAF). Oil loading ranged from 0.05-1 g/L. Newly-hatched larvae of mummichog, *Fundulus heteroclitus*, were exposed in a static renewal assay for 96 h, at 20°C. The effects on body length and ethoxyresorufin-*O*-deethylase (EROD) activity and their relationships to PAH concentrations in water were assessed. At an oil loading of 0.2 g/L, the addition of dispersant caused respectively a 2-fold and 7-fold increase in total PAH (tPAH) and high molecular weight PAH with 3 or more benzene rings (HMWPAH) concentrations. EROD activity was positively correlated to HMWPAH (r^2=0.68, p=0.0002) and not to tPAH. A 5-fold increase in EROD activity was observed in larvae exposed to DCWAF at a loading of 0.05 g/L (tPAH=143 ng/L, 7.4% HMWPAH) while a 4-fold increase was observed with WAF at a loading of 1 g/L (tPAH=244 ng/L, 3.6% HMWPAH). Both DCWAF and WAF caused a slight reduction of body length (5%) which was not correlated to tPAH or HMWPAH concentrations. This study demonstrates that the addition of dispersant increases the risk of toxic effects for early life stage of fish by markedly increasing the water concentrations of HMWPAH.

Pulp mill biosolids: an initial survey of wood extractive constituents and aquatic toxicity. S. Hawkins[1] C. Hedley[2], M.R. van den Heuvel[3] and P.V. Hodson[2]. [1]Indian and Northern Affairs Canada, Iqaluit, NU; [2]Department of Biology, Queen's University, Kingston, ON; and [3]New Zealand Forest Research Institute Ltd., Rotorua, New Zealand.
The use of pulp mill biosolids as a soil amendment on agricultural and silvicultural land is an increasingly prevalent practice. The potential risks of biosolids to aquatic biota may vary with treatment process, wood furnish, and phase of treatment. We sampled the final combined biosolids from thermomechanical (TMP) and bleached kraft (BKM) pulp mills using activated sludge treatment systems, and separate primary and secondary biosolids from an aerated stabilization basin (ASB) system treating the combined effluent of TMP and BKM mills (TMP/BKM). Acute median lethal concentrations (LC50s) were 0.56, 6.4, >18, and >10 g dw/L for TMP, BKM, TMP/BKM primary, and TMP/BKM secondary biosolids respectively. Acute mortality appeared to be generally consistent with biosolid resin acid content, and EROD induction was observed only in those fish exposed to biosolids with high concentrations of retene. Chronic testing with early life stages (ELS) of trout yielded LC50s of 0.26, >10, and >10 for TMP, BKM, and TMP/BKM primary solids respectively. Signs of sub-lethal ELS toxicity included craniofacial malformations, hemorrhaging, and edemas. Toxicity was observed only in samples with high resin acid or retene concentrations, and exposures of ELS to pure dehydroabietic acid or retene indicated that signs of sub-lethal toxicity were consistent between pure compounds and biosolid exposures. Our findings suggest that effluent constituents persist or are biotransformed during secondary treatment, and that the resulting biosolids may continue to pose a toxicity hazard to fish.

Resin acid biotransformation in two pulp mill biosolids. S. Hawkins[1], C.W. Khan[2] and M.R. van den Heuvel[3]. [1]Indian and Northern Affairs Canada, Iqaluit, NU; [2]Department of Biology, Queen's University, Kingston, ON; and [3]New Zealand Forest Research Institute Ltd., Rotorua, New Zealand.
Recent evidence has indicated that high concentrations of resin acids may occur in the solid wastes (biosolids) generated from primary and secondary pulp mill effluent treatment. These biosolids are being increasingly used as soil amendments, and may be stockpiled prior to land application. The biotransformation reactions of resin acids in anaerobic sediments have been well documented, and we therefore now test the hypothesis that resin acid transformation may also occur in biosolid

anaerobic storage or environmental exposure conditions. Biosolids containing resin acids were obtained from two mills, and storage modeled by incubation in dark anaerobic conditions at 4 and 24°C over 32 weeks. Land application was modeled with direct exposure of biosolids to the elements. Following incubation, the concentrations of resin acids, and biotransformation products thereof, where measured in all samples. Biosolids incubated for 32 weeks were also tested for acute toxicity and CYP1A induction potential in juvenile trout. Results of the anaerobic incubations indicated that resin acid biotransformation occurred only at 24°C, and only in biosolids containing secondary (biological) treatment system waste. There were no resin acid neutrals formed in any of the anaerobic incubation treatments, and no evidence of altered biosolid acute lethality or CYP1A induction potential. Outdoor exposures, in contrast, suggested that resin acid neutral formation might occur under environmental conditions. These results suggest that anaerobic biosolids storage does not pose an increased toxicity hazard, however the fate of resin acids in land application scenarios may be an area in need of further investigation.

Blue sac disease in early life stages of white suckers (*Catostomus commersoni*) **exposed to oil sands.** M.V. Colavecchia[1], P.V. Hodson[1] and J.L. Parrott[2]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

White sucker (*Catostomus commersoni*) gametes and juveniles were collected from local tributaries of Lake Ontario. Artificially fertilized embryos were exposed to natural river sediments from the oil sands region of the Athabasca River, Alberta, Canada. Early life stage (ELS) sediment toxicity tests (35 d static renewal) were conducted using controls, natural river bitumen sands, reference sediments and oil refining wastewater pond sediments. 25 fertilized eggs/group were exposed to an exposure gradient of sediments (25.0, 6.25, 1.56, 0.39 and 0.098 g/L), 1 L of water and moderate aeration. Eggs and larvae were checked daily for mortality, hatching and deformities. At various stages of development embryos and larvae were assessed for cytochrome P-4501A (CYP1A) induction using immunohistochemical methods. Growth estimations were determined for larvae at hatch, 7dph, 14dph and 21dph using digitizing imaging. Bitumen-exposed and oil refining wastewater pond sediments groups showed significant exposure-dependent responses in ELS mortality, larval deformities and growth. Deformities included edemas, hemorrhages, spinal malformations and craniofacial alterations. Juveniles exposed to natural bitumens and oil refining wastewater pond sediments (96 h) demonstrated significantly increased EROD induction as compared to controls. Reference-exposed groups and water controls demonstrated excellent embryo and larval survival, minimal malformations and negligible CYP1A induction. These observed symptoms of blue sac disease (ELS mortality, malformations, growth reductions, CYP1A induction) may cause deleterious reproductive effects and recruitment failure in natural fish populations exposed to oil sands mixtures.

A special challenge for the Domestic Substances List program: categorizing "unknown or variable composition, complex reaction products and biological materials" (UVCBs). S. Schnabel, D. Caron, P. Doyle, P. Robinson, A. Sene, A. Okonski and A. Au. Environment Canada, Gatineau, QC.

The *Canadian Environmental Protection Act* (CEPA 1999) requires the Minister of the Environment and the Minister of Health to categorize substances listed on the Domestic Substances List (DSL) by September 2006. The criteria used to categorize substances are described in Section 73 of CEPA and identify those substances that (i) "may present, to individuals in Canada, the greatest potential for exposure or (ii) are persistent or bioaccumulative in accordance with the Persistence and Bioaccumulation Regulations, and inherently toxic to human beings or to non-human organisms, as determined by laboratory or other studies." The DSL includes 23000 substances nominated to Environment Canada that were in Canadian commerce between January 1, 1984, and December 31, 1986. The DSL contains many different chemical classes and groups, including organic and inorganic compounds, polymers, and UVCBs (Unknown or Variable composition, Complex reaction products and Biological materials). Environment Canada, with the assistance of a contractor, developed a preliminary strategic approach for grouping and categorizing UVCBs. A total of 4235 UVCBs have been grouped in six "blocks": organics, inorganics, organometallics, polymers, biologicals, and others (unknown and variable composition substances). Further grouping will be performed considering UVCB's chemical nature and properties, and a moiety-of-concern approach. Based on in depth searches, Environment Canada has determined that UVCBs are data-poor. Only a few international jurisdictions address in some manner this type of substance (US EPA, OECD). Multiple questions have arisen while reviewing the potential persistence, bioaccumulation and inherent toxicity of these substances. UVCBs therefore represent a new and special challenge for categorization. The current strategic categorization framework will be presented.

CYP1A inducing compounds in crude oil. C.W. Khan, B. Hollebone, S. Ramachandran, L.M. Clarke, Z. Wang and P.V. Hodson. Department of Biology, Queen's University, Kingston, ON.
CYP1A induction, as measured by the EROD assay, is an effective biomarker of polycyclic aromatic hydrocarbon (PAH) uptake by fish. Crude oil is a major source of PAH in the aquatic environment. PAH are known to cause developmental malformations in the early life stages of fish and we have observed similar effects in larval fish exposed to crude oil in the laboratory. Due to the complex chemical make-up of crude oil, it is uncertain as to which constituents cause toxicity. This research is part of a Toxicity Identification and Evaluation (TIE) of different crude oils, to test the hypothesis that the toxicity of crude oil is due primarily to the PAH. The aim of the current experiment is to compare the amount of bioavailable PAH among different crude oils, and to demonstrate whether chemical fractionation of these oils separates PAH and toxicity in the same fractions. Bioavailable PAH were measured by the extent of CYP1A induction in rainbow trout exposed to oil in water over 48 h. Fractions of each oil were created by low temperature distillation, corresponding roughly to white gas, kerosene, coal tar/bitumen, and wax. Preliminary results show that whole oil induced hepatic EROD activity with an increase in exposure concentration. We expect that the coal tar/bitumen fraction, the highest in PAH, will account for all observed CYP1A induction in the whole oil.

Environmental effects monitoring (EEM) - pulp and paper, "the unabridged version/ Étude de suivi des effets sur l'environnement (ESEE) - pâtes et papiers, "La version intégrale"

Session Co-chairs/Présidents: J.M. Culp and/et T. Kovacs

10 Years of EEM for pulp and paper – we've come a long way! K. Hedley. Environment Canada, Environmental Quality Branch, Gatineau, QC.
In 1992 the *Pulp and Paper Effluent Regulations* (PPER) were promulgated under the authority of the *Fisheries Act*. The PPER were the first regulations in Canada to require that users of Canadian natural resources monitor the receiving environment to help to determine if the national baseline regulations are providing adequate environmental protection. The Canadian EEM was new and innovative, and remains globally unique. As with any new and innovative programs, problems were encountered with the implementation of the EEM program. The annual Canadian Aquatic Toxicity

Workshop has been, and remains, a key forum for discussion on EEM. Stakeholders worked together to overcome the initial difficulties experienced with the EEM program, and the hard work has paid off with program now generating reliable results that can be used for regional and national analyses. But there is still more to do. This presentation will provide an Environment Canada perspective on where the EEM program has come from in the past ten years, but more importantly, where the EEM program needs to go to over the next 10 years and how we need to work together to get there.

A 10 year industry retrospective on the EEM program. R. Cook[1] and I. Cloutier[2]. [1]Forest Products Association of Canada, Ottawa, ON; and [2]Abitibi-Consolidated Inc., Montreal, QC.
Established in 1992, the Environmental Effects Monitoring (EEM) program required pulp and paper mills to conduct studies on receiving waters in order to assess and monitor effects potentially caused by effluent. Canada is the only country in the world to have required industry to implement a program like EEM, and pulp and paper is the first industrial sector in Canada to have developed a national EEM program. So far Canadian pulp and paper mills have invested more than $18 million to monitor the effects of mill effluents on the aquatic environment. Since the EEM program's inception in 1992, mills have completed two four-year sequences of monitoring and interpretation phases known as "cycles" and are currently into the third cycle. Cycle 1 covered the period 1992 to 1996 and Cycle 2 the period 1996-2000. Cycle 3 started in 2000 and will end in 2004. This presentation will focus on the challenges faced by the industry in the early development stage of the program, the difficulties encountered during the first two cycles and the challenges that still remain with the program. The presentation will also describe the constructive aspects of the program, highlight industry's issues with the current structure and articulate industry's concerns on the future of the program.

Environmental effects of pulp and paper mill effluents in Canada following regulatory changes made in 1992. M.E. McMaster, J.L. Parrott and L.M. Hewitt. Environment Canada, National Water Research Institute, Burlington, ON.
In Canada, new federal regulations were passed in 1992 under the *Canadian Environmental Protection Act* (CEPA) to control releases of dioxins and furans. As well, the existing *Pulp and Paper Effluent Regulations* under the *Fisheries Act* (FA) were revoked and replaced by an updated set of regulations. The new FA regulations set stricter limits for biochemical oxygen demand (BOD) and total suspended solids (TSS), while maintaining a similar non-acute lethality requirement. Unlike the 1971 regulations, the limits in the new regulations became legally binding on all mills. The new regulations included requirements for Environmental Effects Monitoring (EEM) at all mills. This provision would allow the effectiveness of the control limits in protecting fish, fish habitat and man's use of fish to be assessed. Research studies were also undertaken by government, industry and academia to examine the effects of pulp and paper mill effluents on receiving environments throughout Canada. This paper describes our 10 year review of effects of pulp and paper mill effluents in Canada following the regulatory changes made in 1992. It includes an assessment of research related to the effects of effluents on wild fish populations, research on the development of laboratory tests to evaluate effects on fish, as well as studies conducted examining potential chemicals responsible for the reproductive effects in fish populations.

Tracking the outcome of environmental investments in the 1990s: a case study. P.H. Martel, T.G. Kovacs and R. Voss. Pulp and Paper Research Institute of Canada, Pointe Claire, QC.
During the 1990s, pulp and paper mills made substantial investments to conform with revised effluent regulations and as part of a general commitment to improve environmental performance. The outcome of these investments was monitored for three mills on the St. Francois River in Quebec. The mills

upgraded or installed secondary treatment systems and also made some process changes. To gauge effluent quality, the monitoring included laboratory sublethal tests required for the environmental effects monitoring (EEM) program. To assess the actual status of aquatic organisms in the river itself, the monitoring included a fish community study as well as caged bivalves. The laboratory sublethal tests showed clear improvement in effluent quality. Similarly, the fish communities downstream from the mills improved substantially. The caged bivalve approach indicated some local effluent-related effects downstream from one mill. The major factor responsible for the overall improvements appeared to be the installation or upgrade of secondary treatment plants.

Reducing uncertainties in pulp and paper EEM by using mesocosms and caged bivalves. M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.
Several uncertainties exist in pulp and paper EEM because of the emphasis on the adult fish survey and the lack of utilizing approved alternatives such as mesocosms and caged bivalves. Many scientists remain skeptical of the fish results. Each of these approaches has distinct advantages and disadvantages, and it is inappropriate to consider them as optional rather than complementary tools. As EEM has evolved, more emphasis has been placed on characterizing and understanding processes to reduce the uncertainties associated with current approaches. Mesocosms and caged bivalves are well suited to answering these questions because they represent controlled experiments in the field as opposed to the observational data from collecting wild fish. We suggest a change in philosophy in EEM to reduce the uncertainty even further by including the following: emphasizing risk assessment-based monitoring, not exposure- or effects-based monitoring; utilizing gradient designs rather than comparisons with reference sites; and increasing the use of accepted and standardized alternative monitoring tools, particularly those that have been under utilized in Environment Canada's EEM program; i.e., mesocosms and caged bivalves. We believe that this shift in emphasis will be necessary to move beyond current approaches that emphasize either exposure-based or effects-based monitoring and toward characterizing and understanding processes associated with exposure, dose, and response and the eventual prediction of effects based on available monitoring data.

A comparison of the fish survey and caged bivalve approach for pulp and paper EEM. S.D. St. Jean[1], S.C. Courtenay[2] and W.R. Parker[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; and [3]Environment Canada, Environmental Protection Branch, Fredricton, NB.
The *Pulp and Paper Effluent Regulations* (PPER) under the *Fisheries Act* require industry to conduct environmental effects monitoring (EEM) to assess effects of their effluent. Recently, caged bivalve studies have been approved as an alternative to wild fish studies. This experiment investigated and compared the potential of these two approaches for PPER-EEM. Five cages of 20 juvenile blue mussels (*Mytilus edulis*) were deployed at each of 5 sites along a distance gradient from a pulp mill effluent, in Boat Harbour NS, and 3 reference sites in June 2002. The cages were exposed for a period of 90 d and the standard EEM measures and supplemental immune measures were taken before and after the exposure. In May 2002, sampling for the fish survey portion of the EEM program was completed following the Environment Canada technical guidance document. A minimum of 20 male and 20 female mummichog fish (*Fundulus heteroclitus*) were sampled from the mill effluent receiving environment and two reference sites. The following measures were taken: age, total length and weight, liver weight, gonad weight, and egg weight and size of at least 20 eggs per female. The results showed that the two EEM approaches both showed effects of PME exposure and that the results were complementary. For the caged mussels, immune endpoints were the most sensitive while the gonadal somatic index (GSI) was the most sensitive endpoint for the fish survey.

Advances in study designs and measurement endpoints used in the environmental effects monitoring program. R.B. Lowell[1] and J.M. Culp[2]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; and [2]Environment Canada, National Water Research Institute, Fredricton, NB.

The Environmental Effects Monitoring Program has undergone numerous changes over the last decade to take advantage of recent advances in aquatic and monitoring science. These improvements have included several aspects of study design, including sampling methodology, location and number of sampling stations, species selection, choice of measurement endpoints, and the use of supporting measurements and alternative approaches. This presentation focuses on a few selected examples of improvements to study design based on the available literature and, in particular, on what has been learned from a national assessment of findings from the pulp and paper program. For example, gradient sampling designs have been included as an additional option. Gradient designs have several advantages, including the evaluation of dose-response relationships in the field. On the other hand, they can also have disadvantages, including a reduced ability to detect effects relative to control/impact designs, a problem that may be lessened by more effective placement of sampling locations. Different measurement endpoints have also been compared, revealing the high sensitivity of a Bray-Curtis measure incorporating changes in community composition. These comparisons have further shown an advantage to using evenness rather than diversity as a key measurement endpoint. Recommendations are provided for additional improvements to study design during future cycles of the program.

Development for investigation of cause in pulp and paper environmental effects monitoring. L.M. Hewitt[1], M.G. Dubé[2], S. Ribey[1], K. Hedley[3], J.M. Culp[4], D.L. MacLatchy[5] and K.R. Munkittrick[5]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]Environment Canada, Environmental Quality Branch, Gatineau, QC; [4]Environment Canada, National Water Research Institute, Fredricton, NB; and [5]Department of Biology, University of New Brunswick, St. John, NB.

Environmental Effects Monitoring (EEM) has completed two cycles in the pulp and paper sector and is initiating its first cycle for metal mining. We have composed a framework that utilizes a tiered approach to investigating cause using research projects recently completed and current projects. A determination of cause ultimately ends with the confirmation of individual chemicals present in a effluent that elicit the responses observed in conducting EEM studies. It is our contention that this level of detail is not required at all sites where differences have been noted, nor is it economically feasible. We propose that the depth to which an investigation of cause is conducted be determined on a site-specific basis which is decided upon by stakeholders. Our framework consists of 3 assessment tiers, each of which provides more information on the sources and identities of the responsible compounds. The first tier of our approach consists of response characteristics that are indicative of the type of stressor. The second tier involves investigating individual process effluents within the industrial facility to determine their relative contributions to the effects that have been observed. The final tier involves isolating and characterizing the individual chemicals associated with the responses using techniques such as bioassay-directed fractionation. This framework can be used in a stakeholder decision-making process to determine the extent of the investigation. Intensive research projects utilizing these approaches at selected sites are providing a database of causative processes and/or substances that will be useful in conducting investigations at other sites and will provide information for guidance documents.

Assessing effects of pulp and paper mill effluent using a modified fathead minnow (*Pimephales* *promelas*) **bioassay**. C.J. Rickwood[1], M.G. Dubé[1], D. MacLatchy[2], J.L. Parrott[3] and L.M. Hewitt[3]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Department of Biology, University of New Brunswick, St. John, NB; and [3]Environment Canada, National Water Research Institute, Burlington, ON.

A short-term reproductive bioassay was modified to observe performance of pair-breeding fathead minnow (*Pimephales promelas*) before and after exposure to treated pulp and paper mill effluent. The reproductive performance of *P. promelas* were firstly assessed over a three week period under control laboratory conditions to obtain a baseline data of various endpoints including cumulative and mean egg production, number of spawning events, hatching success and occurrence of deformed larvae. At the end of the pre-exposure, breeding pairs were exposed to 50% and 100% concentrations of treated effluent for an additional three weeks at which time pre- and post- exposure data were compared. It was evident that the number of spawning events and hatching success were reduced and increased occurrences of deformed larvae were observed at 100% exposure compared to controls. Obtaining pre-exposure data and the use of pair-breeding fathead minnow in this assay gave a sensitive indication of effluent effects on reproductive performance. Development of this bio-assay for use in artificial streams *in situ* is currently underway.

Gonadosomatic index for caged blue mussels (*Mytilus spp.*): **Pacific and Atlantic comparison.** S.D. St. Jean[1], P. van Pollelen[2], K.K.J. Kim[3], F. Bishay[2] and S.C. Courtenay[4]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Greater Vancouver Regional District, Burnaby, BC; [3]Environment Canada, Environmental Protection Branch, Dartmouth, NS; and [4]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB.

The *Pulp and Paper Effluent Regulations* requires the industry to conduct EEM studies to assess effects of effluent. The results from Cycles 1 and 2 EEM studies have indicated problems with wild-fish studies which has lead to caged bivalves and mesocosm studies to be approved by Environment Canada as alternatives to assess the effects of effluent on fish. The national assessment of fish data indicated that for most of the results for Cycles 1 and 2, the reproductive system may be negatively affected by pulp mill effluent and requires more attention. The endpoints for the Environment Canada approved caged bivalve studies currently do not include an endpoint to assess the effects of effluent on reproduction and much effort has been directed towards developing one. This presentation describes two simultaneous caged bivalve studies on the East and West coast of Canada and the development of the reproductive index for mussels, equivalent to the gonadosomatic index (GSI) for finfish. We will discuss the technique/method and the utility of this measure on both the Pacific and Atlantic coasts. There are a number of differences between the two coasts which will affect this index. These include differences in water depth, seasonal temperature, response patterns, and prevalence of different *Mytilus* species. Differences between the prevalent species potentially result in differences in the reproductive index for normal healthy mussels. The implications of these differences to a formal environmental monitoring program will be discussed.

Proteomic profiling of abietic acid exposure in rainbow trout cell lines. L.E.J. Lee, S.K. Wagg, S.B. Willfang, P. Ibrahim and M.S. Allen. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Abietic acid (AA) is a wood derived chemical commonly found in pulp and paper mill effluents. AA has been shown to be toxic to a variety of aquatic and terrestrial organisms and has been implicated as an endocrine disruptor. The effects of AA at the cellular level have not been studied in detail and this study aims to research the effects of AA at the cell proteome level. Comparison of protein profiles whose expression is modulated by toxicant exposure could provide insights into mechanisms of aquatic toxicant action and help develop new biomarkers. In mammals, this approach has led to the development of various biomarkers that have improved toxicant screening, diagnosis and treatments. Comparison of cellular proteomes using cell lines has facilitated evaluation of such changes because of their ease of handling and manipulation. The study of toxicant exposure in fish could similarly be enhanced if suitable models and bioindicators became available. Towards this goal, the rainbow trout cell lines, RTG-2 (gonadal fibroblasts), RTL-W1 (liver epithelial cells) and Rtgill-W1 (gill epithelia) were evaluated for visible differences in their proteome with and without exposure to AA, as a sample toxicant. Analysis of 2D gels from control and AA treated samples showed distinct differences in protein spots that could be used as signature profiling of toxicity.

Environmental effects monitoring of pulp and paper mills in Quebec: summary of toxicity for the period 2000-2003 (six first series of Cycle 3). M. Mikhail, I. Matteau and C. Langlois. Environnement Canada, Québec Region, Montréal, Qc.
As part of the Environmental Effects Monitoring (EEM) done in accordance with the federal *Pulp and Paper Effluent Regulations*, 41 mills discharging their effluents in freshwater must twice a year (January-April and July-October) subject their effluent to three toxicity tests: growth of the green algae *Selenastrum capricornutum*, survival and reproduction of the water flea *Ceriodaphnia dubia* and survival and growth of the fathead minnow (*Pimephales promelas*). The results compiled in this study are based on five different samples per effluent and cover the period of summer 2000, winter and summer 2001 and winter and summer 2002, for a total of 205 analyzed samples. A large variability is still present in the test results, between the mills on the one hand, and for the same effluent during the various sampling periods on the other. Data will be analyzed by the type of effluent treatment and process. Results will also be compared with the Cycle 2 data.

EEM - metal mining / ESEE - mines de métaux

Session Co-chairs/Présidents: N. Ali and/et G. Watson

Baseline EEM program for the Tulsequah Chief Mine - northwestern British Columbia. D. Osmond and F. Pearson. Gartner Lee Ltd., Burnaby, BC.
An aquatic environmental effects monitoring program (EEM) for the Tulsequah chief mine was conducted in 1998 and 1999. The purpose of the 2 year program was to collect reliable background data against which potential construction and operations effects at critical water resource locations associated with the proposed mine site, could be compared. Study objectives were to establish aquatic baseline conditions in potential mine site receiving waters and determine pre-construction natural variability for critical physical, chemical, and biological parameters that could be affected by mine operations. Related to these objectives, was the determination of reasonable approaches and threshold responses that would ensure detection of impacts in the aquatic ecosystem, leading to corrective actions by the mine. Sampling was undertaken at a total of 7 surface water sites plus 4 groundwater locations including 1 artesian site where upwelling was evident. Data collected included stream flow; surface water, groundwater, and sediment chemistry; benthic invertebrates; and periphyton. Findings from the pre-construction baseline work included the common detection of metals in surface waters and sediments, and cyanide in groundwater at levels above Provincial Water and Sediment Guidelines for aquatic life. Benthic data indicated good consistency from year to year in types and mix of organisms, although in 1 tributary, numbers of organisms were markedly higher in 1999. Power analysis provided helpful guidance related to strengthening the database.

Metals and metallothionein in fish from a freshwater system receiving gold mining effluents. V.P. Palace, C.L. Baron, R.E. Evans, S. Kollar, J. Werner and K.L. Wautier. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

White suckers (*Catostomus commersoni*) were captured during 3 successive years from Balmer Lake, a shallow freshwater system in Central Canada that has served as the final repository for tailings from two gold mines for more than 40 years, and from nearby reference locations. Concentrations of As, Se, Hg, Cd, Cu, Ni, Pb and Zn were measured in liver, kidney and gill tissues. Enrichments of several metals were identified in the fish captured from Balmer Lake relative to the reference sites. Concentrations of the metal binding protein, metallothionein, were also measured in liver tissue of fish from Balmer Lake and the reference locations, in order to examine relationships between metallothionein concentrations and any of the analyzed metals. The results of these studies show the potential for accumulation of several metals/metalloids in fish exposed to effluent from gold mining. They also demonstrate the potential for metallothionein to be used as an indicator of exposure to metals.

The effects of metal ion exposure on α-Synuclein aggregation in the nervous system of fish. G. Ross[1], H. Ross[1], J. Rossiter[1], L. Williams[1] and V.P. Palace[2]. [1]Department of Physiology, Queen's University, Kingston, ON; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Parkinson's disease (PD) is a late-onset neurodegenerative disease likely caused by a combination of genetic and environmental risk factors. Pathologically, PD is characterized by a loss of dopaminergic neurons originating in the substantia nigra, and abnormal intracellular aggregations of á-synuclein protein. The formation of these deposits can be induced by a number of toxicants, including metal ions. Fish provide a useful model to study the long-term biological effects of metal ion exposure, but no studies have been reported concerning such exposures and á-synuclein aggregation. White suckers (*Castotomus commersoni*) were sampled from the Red Lake area of North-Western Ontario, a region highly contaminated by metal ions due to mining activity. Central nervous system (CNS) samples from fish from two test sites, Balmer Lake and Bug River, were immunolabelled using an antibody against human á-synuclein protein, which positively identifies á-synuclein aggregates. We have observed á-synuclein-like immunoreactivity within the cytoplasm of neuronal populations in exposed fish, potentially reflecting metal ion exposure leading to CNS toxicity. These findings demonstrate that fish are an important new model for studying environmental risk factors and PD. (Supported by the Friends of Parkinson's Research and the Queen's University Botterell Foundation).

Comparison of a partial life cycle bioassay in artificial streams to a standard beaker bioassay to assess metal mine effects on *Chironomus tentans*. K.A. Hruska[1], M.G. Dubé[1] and G. Watson[2]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; and [2]INCO Ltd., Copper Cliff, ON.

There is a need to develop more environmentally relevant bioassays to assess the effects of complex effluents on aquatic biota. A novel, partial life cycle bioassay using *Chironomus tentans* in artificial streams was developed for evaluating the effects of metal mine effluents. The utility of this bioassay was compared to an existing beaker life cycle bioassay. *C. tentans* larvae were exposed to 45% treated effluent from the Copper Cliff Mine (INCO Ltd., Sudbury, ON) from day 11 through to hatching of the second generation. Response patterns were consistent between the two bioassays for fecundity and hatching success. However, patterns of response of *C. tentans* to the effluent differed between the bioassays for survival and sex ratios. Significant effluent effects were measured for survival in the artificial stream bioassay but not in the beaker bioassay. Conversely, significant sex ratio effects were observed in the beaker bioassay but not in the artificial stream bioassay. These differences may be a consequence of the number of organisms that can be tested in each of the systems. These results provide evidence that the bioassay in artificial streams can be an effective tool for evaluating the effects of complex metal mine effluents on life cycle endpoints in *C. tentans*.

Acute toxicity of sulfur oxyanions to ***Daphnia magna*** **and** ***Selenastrum capricornutum*****.** M.L. Schwartz, J.C. McGeer, M.M. King, S. Brigham and J.C. Nadeau. Natural Resources Canada, CANMET-MMSL, Ottawa, ON.

The processing of sulfide ores can result in the formation of sulfur oxyanions which may be released into the aquatic environment. These partially oxidized anions, or thiosalts, can occur in mine effluents at concentrations up to 700 mg/L. Although metals are expected to be the primary cause of toxicity in mine effluents, thiosalts have the potential to contribute to toxicity both directly as well as indirectly via a pH depression. Generally, thiosulfate, trithionate and tetrathionate account for the greatest proportion of total thiosalts in mine effluents and are therefore the most relevant species to determine potential toxicity to aquatic biota. We determined the toxicity of thiosalts both alone and in mixtures to *Daphnia magna* and *Selenastrum capricornutum*. Trithionate and, to a lesser extent, tetrathionate were found to be unstable in solution. Thiosalts were more toxic to *D. magna* than to *S. capricornutum* (thiosulfate and tetrathionate L(E)C50s were ~7 and ~2 higher for *S. capricornutum*, respectively). For *D. magna*, the relative toxicities of thiosalts were thiosulfate <tetrathionate <trithionate. In mixture experiments, important antagonistic effects were identified for all combinations of anion pairs thus having significance in identifying sources of toxicity in mine effluents. Our results indicate that, in the context of mine effluent, direct toxicity is a potential issue only for thiosulfate and tetrathionate to *D. magna*. However, indirect toxicity related to pH degradation is a potential issue for both *S. capricornutum* and *D. magna*.

Aquatic moss (*Fontinalis antipyretica*) **21 day growth bioassay.** J. Pickard[1] and T. Davies[2]. [1]BC Research, Vancouver, BC; and [2]Department of Civil Engineering, University of British Columbia, Vancouver, BC.

Mosses can also be used as bioindicators of environmental contaminants because they lack a well-developed cuticula and vascular tissue which makes them sensitive to environmental pollutants. Relatively rapid uptake kinetics have been observed in mosses providing reliable responses over relatively short exposure periods (1-10 d). A 21 d test was designed to determine the effects of pollutants on growth, as measured by dry weight, shoot growth and chlorophyll levels. The aquatic moss, *Fontinalis anitipyretica* was selected as it is one of the most widely distributed Fontinalaceae species and has wide distribution in British Columbia. Two static-renewal tests were conducted. One test was designed to determine the toxicity of sulphate on the moss. The lowest observable effect (LOE) on chlorophyll levels and shoot length was 400 mg/L sulphate. The LOE for dry weight was 600 mg/L sulphate. The other test examined the toxicity of a mine effluent using receiving water as a diluent. The highest concentration tested was a 1:1 mixture which showed no effect on any of the three endpoints. In conjunction with the second test, five successful reference toxicant tests were conducted using $CuCl2•2H2O$. Shoot death, e.g., brown colour, was evaluated as an acute endpoint of these tests. The mean of the five LC50 values was 134.31 μg Cu/L. These results show that this species of moss can react in a predictable manner to a known toxicant, and can be used to assess the potential for adverse effects of mine effluents and metals on aquatic mosses.

Culturing of metal mining EEM toxicity testing organisms. C.A. Huras. ASI Group Ltd., St. Catharines, ON.

New legislation by Environment Canada, such as *Metal Mining and Pulp and Paper Effluent Regulations* coupled with provincial requirements has resulted in a marked increase in demand and frequency of toxicity tests to comply with these regulations. As a result, it has become more essential than ever for laboratories to maintain healthy test cultures in ample supply to complete the required toxicity tests. In particular, the *Ceriodaphnia dubia*, Fathead minnow and *Selenastrum capricornutum* tests have been performed in commercial labs for quite some time, but there are now larger demands on the in-house cultures needed to initiate these tests. These demands can create new difficulties and challenges in the culturing of these organisms. The *Lemna minor* protocol is more recent and will be put to trial in the next few years with numerous laboratories performing the test for many different mining operations. The initiation of a new testing program at a commercial lab normally requires some problem solving to properly culture and maintain the organisms. Beginning a *L. minor* testing program is no exception. The key to producing accurate, valid and reproducible test results is to begin with optimally healthy cultures. Planning ahead and carefully monitoring organism health play an important role in this process. This presentation will discuss some of the difficulties that may be encountered in the culturing of toxicity testing organisms and procedures to ensure that appropriate numbers of healthy organisms are available to initiate these tests.

Repeated measures (RM) ANOVA and regression designs for environmental effects monitoring programs. M.D. Paine. Paine, Ledge & Associates, North Vancouver, BC.
ANOVA designs compare chemical or biological variables (Y) among discrete areas, with n replicate stations within each area. Regression or gradient designs assess the relationship between Y variables and some measure of anthropogenic stress (X; e.g., distance from a source). The n sample stations represent n values of X, and replication within stations is not required, and rarely cost-effective. In Repeated Measures (RM) designs, the same replicate stations are re-sampled over time. RM designs are most powerful when carry-over effects (i.e., persistence of natural spatial differences over time) are strong. RM designs will be illustrated by case studies. In Environmental Effects Monitoring Programs, RM or re-sampling is rarely used in ANOVA designs when it could be, and statistical analyses appropriate for RM are rarely used in regression and similar designs, although the same stations are often re-sampled over time.

Using artificial streams to assess the effects of metal mine effluent effects on the lifecycle of the freshwater midge (*Chironomus tentans*). K.A. Hruska[1], M.G. Dubé[1] and G. Watson[2]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; and [2]INCO Ltd., Copper Cliff, ON.
In 2002, we developed a life cycle bioassay with *Chironomus tentans* in artificial streams to evaluate the effects of a complex metal mine effluent under ambient environmental conditions. The bioassay was tested in the field using effluent from the Copper Cliff Waste Water Treatment Plant at INCO Ltd. in Sudbury, ON. *C. tentans* was exposed throughout its life cycle to 45% Copper Cliff effluent, which is an environmentally relevant concentration. Effluent-related effects included decreased survival, decreased total emergence, increased time to emergence, and decreased hatching success relative to a reference water treatment. There was no significant effect of exposure on growth, sex ratios, number of egg sacs per female and number of eggs per egg sac. This research evaluated how a life cycle bioassay could be used *in situ* to assess metal mine effluent effects on a soft-sediment benthic invertebrate.

Use of small bodied fish species in EEM programs: benefits and costs of age determination. P. Orr[1], C. Russel[1], J. Tost[2] and R. Pedlar[1]. [1]Minnow Environmental Inc., Mississauga, ON; and [2]North Shore Environmental Services, Thunder Bay, ON.

The use of small bodied fish species in environmental effects monitoring (EEM) programs is becoming more common because smaller bodied fish are usually more abundant, easier to capture and more sedentary than larger bodied fish. However, they also pose some disadvantages; there is usually less known about their basic biology, they can be more difficult to process due to their smaller size, and a single appropriate aging structure has not been determined for most species. Mimic shiner (*Notropsis volucellus*) and Johnny darter (*Etheostoma nigrum*) were used as sentinel species in two Cycle 3 pulp and paper EEM programs conducted in 2002. Multiple structures (otoliths, scales and fin rays) were taken from each species in order to assess which structure was most suitable for age determination. This presentation will examine the usefulness of determining ages of small bodied fish species for EEM programs based on the benefits and cost associated with age assessment. First, the difficulties encountered when working with each of the structures will be discussed. Second, the ages determined from the different structures will be compared for each species. Lastly, the implications for statistical analyses involving fish ages will be discussed.

Ecological risk assessment of metals in the environment / Évaluation des risques écologiques des métaux dans l'environnement

Session Co-chairs/Présidents: P.M. Chapman, P.G.C. Campbell and/et R.R. Goulet

Metal sources for freshwater invertebrates: pertinence for risk assessment. L. Hare et A. Tessier. Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Sainte-Foy, Qc.
Ecological risk assessments are likely to be more effective if they are built upon knowledge of "from where" and "in what manner" animals take up contaminants. We discuss the relative importance of various metal sources for aquatic invertebrates. First, we address the question do sediment-dwelling animals take up their metals from the overlying water compartment or the sediment compartment (both compartments include water and particles). We find that the overlying water column is more important as a metal source for many insects, whereas the sediment compartment is more important for oligochaete worms. We explain this tendency by the behaviors of the animals involved. Second, we ask the question do animals take up their metals from food or water within a given compartment. We conclude that, for some predatory insects, food is their major source of several metals. Thus, ignoring food as a metal source could severely underestimate metal exposures for such animals. We suggest that integrating these complexities into laboratory tests and risk assessment protocols will improve their realism and thus their ability to protect aquatic ecosystems.

Using bivalve tissue copper residues and effects for ecological risk assessment. M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.
In aquatic biota, tissue residues may be a more appropriate indicator of adverse effects than external water concentrations because tissue residues represent a more toxicologically relevant "dose." The tissue residue-effects paradigm is combined with an effects-range paradigm to develop tissue residue guidelines that can be used for ecological risk assessment. Cu in marine and freshwater bivalves is used as an example because there are more dose-response data for Cu than any other substance. Based on 43 studies with marine bivalves, the mean effects concentration (EC-tissue) is 80.3 mg/kg dw and the mean no effects concentration (NOEC-tissue) is 23.9 mg/kg dw. Although only 13 dose-response studies have been conducted with freshwater bivalves, the predicted critical body residues (CBRs) are similar at an EC-tissue of 64.3 mg/kg and an NOEC-tissue of 26.5 mg/kg dw. There are two CBRs for Cu in bivalves because bivalves are partial regulators of Cu. CBR-1 occurs at a threshold

concentration above which bivalves no longer regulate and begin to accumulate higher concentrations of Cu in their tissues. CBR-2 occurs at a threshold concentration above which bivalves cannot accumulate higher concentrations of Cu. The ultimate objective of the tissue residue-effects paradigm, which should be used as a component of ecological risk assessment, is to predict and diagnose the causes of biological and ecological effects.

Bioaccumulation of metals by *Hyalella azteca*: which models best describe accumulation? W.P. Norwood[1], D.G. Dixon[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.
Total metal concentrations in the environment do not provide accurate estimates of toxicological effects since toxicity is a function of metal speciation and bioavailability. Biological effects are usually a function of bioaccumulation. Whole body or tissue concentrations should be a better predictor of effects than concentrations in field-collected sediment and water samples. The Biotic Ligand Model (BLM) approach to predicting toxicity assumes that toxicity is a function of metal bioaccumulation as affected by the concentration of the metal and competing cations in the water. The relationship between bioaccumulation and water concentration was determined in laboratory controlled experiments using the freshwater amphipod *Hyalella azteca*. The allometric model, historically used to describe metal bioaccumulation in laboratory exposures, was compared to a saturation model. Saturation models are more consistent with the BLM approach in describing metal accumulation. As well 24 h elimination rates were determined and evaluated, so that loss of metal from the body of an organism can be understood. This latter factor is particularly relevant to sediment assessments in which exposed animals must undergo gut clearance prior to metal analyses.

Integrating a metal bioaccumulation model into risk assessment methods: example of cadmium and *Daphnia magna*. R.R. Goulet[1], P.J. Doyle[1] and L. Hare[2]. [1]Environment Canada, Existing Substances Branch, Gatineau, QC; and [2]Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc.
The *Canadian Environmental Protection Act* (CEPA 1999) requires the Ministers of Environment to categorize 2300 metal substances on the Domestic Substances List (DSL) by identifying substances that are persistent or bioaccumulative, and inherently toxic to non-human organisms. As much as 30% of these metal substances could be categorized "in." These substances must undergo screening assessments, to determine if they are toxic as defined in CEPA. Risk analyses typically focus on potential for harm to the most sensitive exposed species such as aquatic invertebrates. In current risk assessment methods, contact with water is considered the main source of metals to aquatic invertebrates, but increasing evidence suggests that metal ingestion from food is important. In this study, we used a dynamic multipathway bioaccumulation model (DYNBAM) to predict Cd levels in *Daphnia magna*. Predicted Cd body burden in *D. magna* were in close agreement with measured values obtained from laboratory experiments. We then estimated potential for harm by comparing predicted metal levels in organisms to metal body burdens associated with chronic toxicity (i.e., reduced fitness). We determined that Daphnia magna population were at risk when free Cd2+ concentration in water was above 0.11 μg/L. This modeling approach is an alternative to the biotic ligand model (BLM). The BLM was validated by short exposures of animals to metals in water only and hence, is more suitable for estimating risk during acute exposure scenarios. Under chronic exposure scenarios, metal uptake from food must be considered and the DYNBAM proposed in this study is more appropriate At present, the integration of the proposed model in ERA methods requires more data (e.g., on metal concentrations in food, assimilation efficiencies, depuration rates, and concentrations in tissue associated with effects) for other metals and organisms. Environment Canada

is looking for opportunities to fill these data gaps.

The role of risk assessment in determining chemistry needs at contaminated sites. G. Gilron, R. Hull, C. Bacigalupo and G. Ferguson. Cantox Environmental Inc., Mississauga, ON.
Analytical chemistry data of site media, including soil, water and sediment, are usually collected prior to the initiation of the risk assessment of contaminated sites. Although these data may suffice for site characterization, they are often inadequate for risk assessment purposes. Through a discussion of several case examples, we propose an alternative iterative approach, whereby the risk assessment drives the design and implementation of chemical characterization of the site. This integrated approach allows the risk assessor to: prioritize those areas of the site that require remediation, help identify data gaps, identify other relevant chemical parameters (e.g., hardness, TOC, Ph), and optimize data collection activities. Through this analysis, we also explore some of the more critical risk assessment issues related to chemical characterization, and their implications on risk assessment results, including: the selection of appropriate method detection limits, total vs dissolved metals in water, whole sediment vs porewater chemistry and other factors influencing bioavailability, metal speciation, and consideration of modifying and confounding chemical factors. This iterative approach also allows for a more focused site investigation process, providing the necessary information for the risk assessment, while resulting in potential cost and time savings over a more expansive site sampling program.

Management of the mercury issue at the La Grande hydroelectric complex, Québec, Canada. R. Schetagne. Hydro-Québec, Montréal, Qc.
Development of hydroelectric reservoirs causes temporary increases in Hg concentrations in fish that may pose health risks to regular fish consumers. In the La Grande hydroelectric complex reservoirs, Hg concentrations in all fish species increased rapidly after impoundment, peaking after 5-9 years in non-piscivorous fishes, and after 10-13 years in piscivorous species, then significantly and gradually declined. Peak concentrations reached levels 3-7 times higher than those measured in surrounding natural lakes. Concentrations in the non-piscivorous species have returned to levels typical of natural lakes 10-19 years after flooding. In the piscivorous northern pike and walleye the rate of decline, which begins after 15 years, strongly suggests that natural concentrations are reached between 20-30 years after flooding. A management program has been implemented to reduce health risks related to Hg exposure. It includes: signing of the James Bay Mercury Agreement; study of the source and fate of Hg in Northern Québec; monitoring of Hg levels in fish and of Cree exposure to Hg; public information campaigns, and fish consumption advisories. Results and reasons for success of this management program will be discussed.

Old fish, small lakes, and high mercury levels in lake trout (*Salvelinus namaycush*) **in northern Canada.** L.M. Doetzel[1], M.S. Evans[2], D.C.G. Muir[3], W.L. Lockhart[4], G. Low[5], J. Delaronde[4], J. Keating[2] and G. Stern[4]. [1]University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]Environment Canada, National Water Research Institute, Burlington, ON; [4]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; and [5]Department of Fisheries and Oceans, Hay River, NT.
High Hg levels have been detected in lake trout, *Salvelinus namaycush*, in many lakes in the Northwest Territories (NT) while other lakes in the same regions have lower levels. This study, through multivariate analysis of a series of lakes in the Mackenzie River basin, is investigating the combination of factors responsible for these variations in Hg levels. By developing a predictive model, we will be able to provide the First Nations communities with advice on probable Hg levels

in their many unstudied lakes. Physical variables include lake area, depth, latitude, and watershed features. Chemical variables include Ph, conductivity, dissolved organic carbon, plant nutrients and particulates. Biological variables include fish age, size, and feeding habits (15-N). Higher Hg levels in lake trout are associated with smaller, DOC-rich lakes with relatively large watersheds. These factors affect Hg transformation to the more readily bioaccumulated methyl mercury. Fish age is important, with lake trout reaching a very old age because of low fishing pressures, and consequently high Hg levels. These old fish also are piscivorous and have acquired more biomagnified Hg than would omnivorous feeding trout.

Mercury alters neurochemical receptor binding characteristics in wild mink (*Mustela vison*). N. Basu[1], M. Gamberg[2], K. Klenavic[3], D. Evans[3], A.M. Scheuhammer[4] and H.M. Chan[5]. [1]Department of Natural Resource Sciences, McGill University, Montreal, QC; [2]Gamberg Consulting, Whitehorse, YK; [3]Department of Environmental and Resource Studies, Trent University, Peterborough, ON; [4]Canadian Wildlife Service, National Wildlife Research Center, Ottawa, ON; and [5]Department of Human Nutrition and Dietetics, McGill University, Montreal, QC.

Piscivorous wildlife, such as mink (*Mustela vison*), are routinely exposed to levels of Hg in their natural environment that may cause adverse behavioral outcomes. The purpose of this study was to characterize possible changes in neurochemistry in an effort to understand the sublethal effects that may precede irreversible health effects. Receptor binding assays for the cholinergic muscarinic acetylcholine (mACh) and dopamine-2 (D2) receptor were conducted in mink brain tissues collected across Canada to calculate receptor density (Bmax) and ligand affinity (Kd), and values were correlated with levels of Hg. Levels of Hg (total and MeHg) were significantly higher in Nova Scotia compared to ones collected from Ontario and Yukon. There was a significant Hg concentration dependent increase in mACh receptor Bmax ($r=0.546$; $p<0.0001$) and Kd ($r=0.413$; $p<0.01$). A stronger correlation was calculated between MeHg and mACh receptor Bmax ($r=0.596$; $p<0.0001$) and Kd ($r=0.474$; $p<0.01$). In contrast to the mACh receptor data, a negative correlation was calculated for total Hg with D2 Bmax ($r=-0.340$; $p<0.05$) and Kd ($r=-0.346$; $p<0.05$). No significant correlation was calculated for MeHg with D2 receptor binding characteristics. Given the importance of the cholinergic and dopaminergic pathways in multiple aspects of behavior, further studies are required to validate the physiological and ecological significance of these findings. Also, neurochemical receptor characteristics represent a novel biomarker of Hg effect in wildlife toxicology.

Hazard and ecological risk assessments of metals, metalloids and inorganic metal substances. P.M. Chapman[1], F. Wang[2], R.R. Goulet[3] and C. Kamunde[1]. [1]EVS Environment Consultants, North Vancouver, BC; [2]University of Manitoba, Winnipeg, MB; and [3]Environment Canada, Assessment Division, Gatineau, QC.

Hazard assessment of metals, metalloids and inorganic metal substances (collectively referred to as metals) should focus on solubility and toxicity rather than persistence and bioaccumulation. Environmental risk assessment (ERA) of metals should additionally focus on natural occurrence, essentiality, speciation and bioavailability. A key consideration is whether or not metals accumulated in biological tissues are in a metabolically available form or in a detoxified and no longer available form. Different information is required for the three different tiers of ERA: Hazard Assessment or Problem Formulation, Screening Level ERA, and Detailed Level ERA. For both types of ERA, there is a need to determine concentrations added to background and exposure duration as well as conducting both deficiency and tolerance tests with organisms pre-acclimated to natural levels of metals. Refinements at the three different tiers relate to increased information and reduced uncertainty for: bioavailability, the predicted environmental concentration (PEC), the predicted no effects concentration (PNEC), and tolerance. Application (uncertainty) factors do not appear to be relevant to metals ERA given current levels of conservatism in the process. Any ERA of metals needs to address three key questions: (i) do metals accumulate in biota above background levels; (ii) are these metals metabolically active; and (iii) if so, are they likely to result in adverse effects.

Geochemistry of lead and chronology of its deposition in remote Canadian shield lakes. C. Gallon[1], C. Gobeil[1], J.C. Nadeau[2] and A. Tessier[1]. [1]Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc; and [2]Natural Resources Canada, CANMET-MMSL, Ottawa, ON.
Dated sediment cores (210-Pb, 137-Cs, 241-Am) from headwater lakes located in uninhabited and undisturbed watersheds were analyzed for Pb stable isotopes, as well as polycyclic aromatic hydrocarbons (PAHs). Porewaters were collected at the same sites by in situ dialysis and analyzed for several constituents including Pb. Equilibrium calculations indicated that dissolved Pb speciation in porewaters was dominated by complexes with natural organic matter. We established a one-dimensional mass conservation equation that accounts for the effects of molecular diffusion, bioturbation and bioirrigation of dissolved Pb. Solving this equation with the assumption of steady state conditions for porewater Pb indicates that post-depositional redistribution of Pb is negligible in all cases and that Pb profiles in the solid phase represent historical variations of Pb deposition. The stable isotope ratios 206-Pb/204-Pb, 206-Pb/207-Pb and 206-Pb/208-Pb are used with total Pb in mass balance equations to determine the contributions of Pb from various sources over the last century, i.e., coal combustion, leaded gasoline combustion, smelter emissions and industrial lead. PAH profiles in the sediments confirm Pb emissions from coal combustion.

The nature of aerosolic particulates in the Sudbury smelter footprint. G. Spiers[1], J. Richard[1], C. Hawson[1] and A. Cheburkin[2]. [1]Centre for Environmental Monitoring, Laurentian University, Sudbury, ON; and [2]University of Heidelberg, Heidelberg, Germany.
The metal mining and smelting industry in the Sudbury, Ontario, area began before the turn of the century, developing into one of the largest metal producing complexes in the world. As important as the 2,000,000 tonnes per annum SO2 emissions in the early 1960s were the 1500 tonnes of metal particulates carried aloft annually in the hot gas plume. The particulates were composed primarily of minute iron-rich fly-ash spherules containing an intergrowth of Ni, Cu and other trace metals. The historic result of the atmospheric washout of this massive release was a denuded landscape with acidified soils contaminated with metals. An extensive and ongoing abatement program has ameliorated impacts significantly, however significant amounts of metal-rich particles continue to be released by the smelter operations within the region. This presentation will describe highlights of current research initiatives describing the nature and distribution of metals and metal-rich fallout particles both in modern ejecta, and as a historical archive in regional soils and wetlands. Data obtained from snow samplers installed in the region of the Sudbury smelter footprint, together with a series along a 150 km NE-SW transect, have provided data describing the dispersion of metal-rich aerosols from the core of the regional smelter district. The aerosol particles were analyzed by a combination of EMMA (Energy-dispersive Miniprobe Multielement Analyzer), XRD, and SEM-EDS.

The speciation of trace metals at the soil:root interface in forest soils. F. Courchesne, P. Legrand, V. Séguin, S. Sauvé et M. Turmel. Université de Montréal, Montréal, Qc.
The characteristics of the rhizosphere, when compared to the bulk soil, include low pH, high organic matter content, intensified mineral weathering and increased microbial counts. The properties of this microenvironment affect macronutrient concentrations and are known to impact the speciation of trace

metals and, hence, their bioavailability. This research program aims at understanding the processes controlling trace metal speciation at the soil:root interface. The objectives are (i) to compare the solid phase fractionation of five trace metals (Cd, Cu, Ni, Pb and Zn), and (ii) to contrast the free Cu++ activity between the rhizosphere and the bulk soil components of contaminated forest soils. Plots were selected along gradients in metal contamination in the Sudbury and the Rouyn-Noranda areas. The rhizosphere material was sampled under various forest covers together with the bulk soil. Five chemical extractants (H2O, BaCl2, Na-pyrophosphate, oxalate and mixed acids) were used to estimate the fractionation of metals. The Cu2+ activity in the solution was measured with an ion selective electrode. The results significantly broaden our understanding of the mechanisms controlling speciation of trace metals in the rhizosphere. Moreover, this work could have a critical impact on the way we conduct ecological risk assessments because it tests the hypothesis that the extent of the functional role of the rhizosphere in the biogeochemical cycling of trace metals is much larger than the volume it occupies in the field.

Risk associated with natural attenuation of trace metal contaminants in soils. W. Hendershot[1], K. Taillon[1], D. MacDonald[1] and S. Sauvé[2]. [1]Natural Resource Sciences, McGill University, Montreal, QC; and [2]Cheme, Université de Montréal, Montréal, Qc.

While it is well known that trace metal contaminants in soils pose a threat to the environment and to human health, it is difficult to judge when it might be better to let nature deal with the problem than to apply an engineering solution. Mathematical equations relating the solubility of Cd, Cu, Ni, Pb and Zn to soil properties (pH, total metal and organic matter contents) have been developed. These equations can be used to estimate the amount of metal that will be mobile in the soil and the length of time it would take for the metal content to decrease to an "acceptable" level. The results show that at low pH the soils will naturally attenuate metals quickly, but the concentrations in the waters leaching out of the soil may lead to toxic concentrations in ground or stream waters. On the other hand, at high pH leaching for a thousand years will not attenuate the metals; however the metals are so strongly held in this situation that the toxicity may be considered too low to be of concern. For each metal there is an intermediate range of pH where the metals will be removed from the soil gradually while keeping the concentrations of metal in the leaching water acceptably low.

Metal competition in the freshwater environment - implications for environmental risk assessment. C.L. Chakrabarti, I.I. Fasfous, T. Yapici and J. Murimboh. Department of Chemistry, Carleton University, Ottawa, ON.

The Biotic Ligand Model (BLM) provides the link between chemical speciation and metal uptake and toxicity. The BLM has accounted for the effects of competition by major cations such as Ca and Mg, which are present at concentrations that are several orders of magnitude greater than the target trace metal. Trace metal competition determines the equilibrium composition, and hence, it is essential for the competing reactions to be fast for rapid equilibration. Other trace metals compete with the target trace metal for the binding sites of dissolved organic carbon (DOC) even when they are present at similar concentrations. Hence, trace metal competition is too important to be ignored if the trace metal competition involves transition metals for which the coordination equilibrium is slow and the equilibrium is not established. The objective of this work was to investigate the influence of metal competition on chemical speciation in the freshwater environment. Competition among trace metals was found to follow the trend predicted by Ligand Field Stabilization Energy (e.g., Mn(II) d5 > Co(II) d7 > Ni(II) d8 > Cu(II) d9 < Zn(II) d10). In our studies on equilibration of spiked additions of Cd(II), Cu(II), and Ni(II) we found that equilibration took at least 24 h. The results suggest that for transition metals with slow coordination equilibrium (e.g., Cu(II) and Ni(II)), the BLM should take into account the kinetics of metal complexation.

Study design for a contaminated foreshore risk assessment: a weight-of-evidence approach. G. Wickstrom and M. Cameron. Keystone Environmental Ltd., Burnaby, BC.
A study design incorporating a weight-of-evidence (WOE) approach for ecological risk assessment was developed to address metal ore and polycyclic aromatic hydrocarbon (PAH) contamination in a northern BC harbour. The contaminants were released to the environment as a result of a fire that occurred in the early 1970s, destroying a foreshore ore distribution facility. Contaminants of concern include As, Cu, Cd, Pb, Ni, Zn and a suite of high molecular weight PAHs. The management objective for the property was to assess and, if necessary, manage risk identified to valued ecosystem components while maintaining the property for industrial use. The study design provides a selection of assessment and measurement endpoints to facilitate completion of the ERA, and to assist with communication of identified concerns. Lines-of-evidence considered in the ERA include soil, sediment, surface water, and combined sewer outfall water quality; bioaccumulation in benthic and soil invertebrates as well as terrestrial and aquatic plants; sediment and surface water toxicity testing; taxonomic benthic community assessment; and completion of terrestrial and aquatic habitat assessments. Hypotheses and decision rules were set *a priori* for each line-of-evidence to ease interpretation of findings. The lines-of-evidence were combined to provide an overall WOE evaluation of the potential for adverse effects to the selected assessment endpoints. Sources of uncertainty for each line-of-evidence were also considered with respect to their potential for effect on the WOE risk characterization.

Development of a bioaccumulation model for a sedimentary trace metal biomonitor. L. Croisetiere, L. Hare et A. Tessier. Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc.
Measurements of trace metals in aquatic animals can allow us to quantify metal exposure and, using an appropriate model, estimate metal concentrations in water or sediment. Such models are most effective when they are mechanistic, that is, they consider both the metal's chemistry and the animal's biology. We used a step-by-step approach to develop mechanistic models relating metal concentrations in larvae of the predatory alderfly *Sialis* to those in its surroundings. Through field and laboratory experiments, we collected key information on the behavior of this insect and the sources of its trace metals. Results from our field experiments suggest that this biomonitor accumulates all of its Cd, Cu and Pb from prey. Furthermore, results of measurements of stable sulfur isotopes in *Sialis* and its surroundings suggest that most of the energy (and likely metals) obtained by *Sialis* comes from the sediment compartment. We incorporated this information into a bioaccumulation model describing the relationship between trace metal concentrations in Sialis and those in its surroundings.

How does research on the invertebrate *Hyalella azteca* fit into environmental risk assessment? W.P. Norwood[1], J. Schroeder[2], M. Nowierski[1], A. Wallace[1], D.G. Dixon[1] and U. Borgmann[3]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Ontario Ministry of the Environment, Etobicoke, ON; and [3]Environment Canada, National Water Research Institute, Burlington, ON.
Accurate and reliable methods of identifying the environmental concentration of metals above which adverse biological effects occur is essential. In the past, environmental quality guidelines have been based primarily on total metal concentrations in water or sediment. These measures do not provide accurate estimates of effects since toxicity is a function of metal bioavailability. Bioavailability is a function of both chemical and biological processes, thus the present research focused on both these

aspects with *Hyalella azteca*. A number of methods have been developed which provide data of significance to ecological risk assessment (ERA). A Biotic Ligand Model for Ni toxicity to *H. azteca* has been developed. This model assumes that toxicity is a function of metal bioaccumulation and competing cations in the water. As well, the relationship between bioaccumulation and toxicity of 10 metals, individually and in mixtures, has been modeled. To complement this laboratory-based research, methods have been developed and tested for field applications. Sediment bioaccumulation and toxicity tests have been developed, from which Toxicity Identification Evaluation was possible utilizing critical body concentrations. Results were compared with benthic community assessments. The results of this study can be used site specifically to identify adverse effects, identify metals of concern, predict effects of bioaccumulated metals, and link laboratory based models to effects observed in the field.

Influence of hydrogen ions on cadmium accumulation by members of a planktonic trophic chain. J. Orvoine, L. Hare et A. Tessier. Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc.
Because hydrogen ions (H+) can compete with free metal ions at biological uptake sites, it is necessary to take into account ambient pH when predicting metal bioaccumulation. This is reported to be the case for Cd concentrations in the phantom midge (*Chaoborus americanus*). However, because this insect takes up its Cd from food (zooplankton), we determined at what trophic levels this Cd2+ - H+ competition occurs. First, we determined whether or not H+ swallowed by *C. americanus* could compete with Cd2+ in its gut lumen. To do this, we held *C. americanus* larvae at constant [Cd2+] but at various pH's (4.5, 5.5, 6.5) and fed them copepods that had been raised on Cd-rich green algae. There was no difference in the Cd accumulated by the predator at the various pHs. To explain this lack of competition, we measured *C. americanus*'s gut pH using dyes. We found that its gut pH remained constant (6.5) even when ambient pH varied (4.5–9.0). Second, we determined if Cd2+ and H+ ions compete at biological uptake sites on *C. americanus*'s prey. To do this, we fed copepods a diet of Cd-rich green algae in the presence of constant [Cd2+] but at various pHs (4.7, 5.5, 6.5). Preliminary results suggest that copepod Cd concentrations are not influenced by ambient pH. If this is the case, then ambient pH only affects Cd uptake by phytoplankton at the base of the food chain leading to *C. americanus*.

Metal detoxification and antioxidant defences in indigenous yellow perch (*Perca flavescens*) **exposed to cadmium, copper, nickel and zinc**. A. Giguère[1], P.G. Campbell[1], L. Hare[1], C. Cossu-Leguille[2], P. Couture[1], G.D. McDonald[3] and J.B. Rasmussen[4]. [1]Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc; [2]Université de Metz, France; [3]Department of Zoology, University of Guelph, Guelph, ON; and [4]Department of Biology, McGill University, Montréal, Qc.
Relationships between metal accumulation and metal-induced effects in aquatic organisms should be improved if the metal body concentrations are expressed not as total metals, but rather in terms of metal subcellular partitioning within particular target tissues. We are testing this prediction with indigenous yellow perch (*Perca flavescens*) collected from lakes with contrasting metal levels but similar water qualities and trophic status. The lakes have been characterized at the geochemical level, to determine the ambient exposure of the fauna to metals. Various cellular fractions were isolated in fish liver by differential centrifugation (granules, nuclei and debris, mitochondria, lysosomes and microsomes, cytosolic enzymes and metallothionein-like proteins), and metal levels were measured in each. We also evaluated lipid peroxidation ([malondialdehyde]) and antioxidant defenses ([glutathione], glutathione-peroxidase and glutathione-reductase activities). Metallothionein-like

proteins appeared important for the detoxification of Cd and Cu. In contrast, Zn and Ni were preferentially accumulated in cytosolic proteins other than metallothioneins, likely metalloenzymes. Lipid peroxidation byproducts decreased with increasing bioaccumulated copper. Site-to-site variations in antioxidants such as glutathione-reductase, glutathione–peroxidase or glutathione did not help explain this hormesis-like response, i.e., antioxidant defences did not appear to be stimulated by metal exposure. The usefulness of metal concentrations in metal-sensitive subcellular compartments as predictors of toxicity will be discussed.

Cadmium dynamics in yellow perch (*Perca flavescens*) **following transplantation to a metal-contaminated lake.** L. Kraemer, P.G. Campbell et L. Hare. Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc.
To study Cd accumulation in indigenous fish under natural conditions, we measured metal uptake rates and hepatic subcellular partitioning in juvenile yellow perch (YP), *Perca flavescens*, that were caught in a reference lake and held in cages in a lake with elevated metal levels in northwestern Quebec, Canada. The mesh size permitted zooplankton, a major source of food for juvenile YP, to freely move in and out of the cages. A 30 d pilot study was carried out in the summer of 2001, followed by a longer study (70 d) in the summer of 2002. Accumulation of Cd was observed in all organs studied (gills, gastrointestinal tract, liver and kidney), but was highest in kidney tissue. However, despite this rapid renal Cd accumulation, concentrations after 70 d were still less than those in the kidneys of indigenous fish from the contaminated lake. Differential centrifugation was used to examine the sub-cellular partitioning of Cd in the liver after exposure in 2002. After 70 d, Cd accumulation decreased in the order: heat-stable fraction (metallothionein) > organelles > heat-sensitive proteins > granules > nuclei and cellular debris. Metallothionein is clearly an important protein for detoxification of Cd in the liver of juvenile YP, but during the 70 d exposure some Cd did accumulate in metal-sensitive cellular fractions, suggesting that metal detoxification is incomplete.

Population and physiological effects of industrial metal contamination in wild yellow perch (*Perca flavescens*). P. Couture[1], J. Rajotte[2] and G. Pyle[3]. [1]Institut National de la Recherche Scientifique - Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc; [2]Department of Zoology, University of Guelph, Guelph, ON; and [3]Department of Biology, Nipissing University, North Bay, ON.
Yellow perch (*Perca flavescens*) were sampled from nine lakes forming two metal contamination gradients around Sudbury and Rouyn-Noranda in 2002 and 2003, respectively, in order to capture the full size range of fish in each lake, and to assess seasonal variability in metal accumulation, morphometric condition, and physiological status. Data collected from fish of each age class included condition, metal concentrations (liver, kidney) and stomach content, and muscle anaerobic and aerobic capacities. Results from 2002 indicated that growth was impaired in yellow perch from metal contaminated lakes relative to those from reference lakes. Although morphometric condition was generally lower in fish from metal contaminated lakes relative to those from reference lakes, condition varied by fish age, gender, and season. Dietary Cd, Cu or Ni, but not Se or Zn, strongly influenced concentrations of the same metals in liver and kidney of yellow perch in both spring and summer. Seasonal and condition-related variations in liver and muscle aerobic and anaerobic capacities were observed and characterized. In addition, elevated tissue metal concentrations, in particular Cd and Ni, were related to lower hepatic cytochrome C oxidase activity, an indicator of aerobic capacity, and to increased muscle anaerobic capacities. By exploiting natural metal contamination gradients and relating physiological effects to environmental metal concentrations, in conjunction with data collected by other members of MITE-RN, this work provides important insights into how a common wild fish

species responds to metals under natural conditions.

Acclimation effects of different dietary factors on metal uptake and toxicity in freshwater fish. S. Niyogi[1], C. Kamunde[2], B. Baldisserotto[3], M. Grosell[4], G.D. McDonald[5] and C.M. Wood[6]. [1]Department of Biology, McMaster University, Hamilton, ON; [2]EVS Environment Consultants, North Vancouver, BC; [3]Department of Physiology, UFSM, Brazil; [4]RSMAS, University of Miami, Miami, FL; [5]Department of Zoology, University of Guelph, Guelph, ON; and [6]Department of Biology, McMaster University, Hamilton, ON.

The long-term overall goals of our research are to use laboratory studies to understand and model the chronic impacts of waterborne and dietary metal exposure (Cu, Cd and Zn) on metal uptake and toxicity in fish, using the rainbow trout as a reference species. For the last two years, our major focus has been to evaluate the influence of acclimation to different dietary factors (e.g., metal content, quality (Na and Ca content)) on both waterborne and dietary metal toxicity. We overview some of the projects carried out in this aspect, illustrating (i) the interaction between waterborne and dietary Cu exposure, (ii) the effects of dietary Na on waterborne Cu uptake and tissue accumulation, (iii) the effects of dietary Ca on waterborne Cd uptake and toxicity, (iv) the interaction between dietary and waterborne Zn uptake, and (v) the effect of dietary Ca on the responses to sublethal waterborne Zn exposure. Our findings have major implications for the future development of chronic biotic ligand models (BLM) in fish, an approach that is gaining widespread interest as a tool for performing aquatic ecological risk assessment of metals.

Aluminum binding to fish gills and to natural organic matter. R.C. Playle[1], J. Nichols[2], A. Winter[1] and S. Smith[3]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [2]Department of Chemistry, Wilfrid Laurier University, Waterloo, ON; and [3]Florida International University, North Miami, FL.

We exposed small rainbow trout (*Oncorhynchus mykiss*) to 3 μM Al in ion-poor water adjusted to pH 4-10. At very low pH Al binding to trout gills was reduced through H+ competition at the gills, even though most of the Al was available in solution as Al3+. The greatest amount of Al bound to the gills was at water pH 6-8; acidification of water in the gill micro-environment by CO2 released at the gills probably increases the availability of Al3+ near the gills at these water pHs. At very basic conditions Al did not bind to the gills, presumably because anionic Al(OH)4- does not bind to the negatively charged gill surface. Suwannee River natural organic matter (NOM; ~5 mg C/L) eliminated Al binding to the gills at all pHs, even though NOM kept more Al in solution at intermediate pHs. That is, NOM reduced precipitation of amorphous Al(OH)3 from the water column by binding Al but reduced accumulation of Al by the gills by the same process. We used two methods to investigate differences in Al binding to NOM collected from different sources (marsh NOM, sewage treatment plant NOM). We first used Al accumulation by trout gills to assess NOM-Al binding differences. We then used changes in excitation-emission scans of NOM as Al binds as an indication of differences in Al binding; these scans act as optical "fingerprints" of NOM from different sources.

Evaluation of parameters regulating phagocytosis in the rainbow trout (*Oncorhynchus mykiss*) **gill cell line, RTgill-W1.** D. Sotornik[1], N.C. Bols[1] and L.E.J. Lee[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Particle clearance from the gills is a normal activity in healthy fish. This process is necessary to maintain optimal gas exchange at the respiratory surface and to serve as a first line of defence against microbial pathogens. The first general step in this clearance process has been attributed to phagocytosis by gill epithelial cells. Impairment of the phagocytic function of gill epithelial cells could have both acute (respiratory failure) and chronic (microbial diseases) impacts on fish. Environmental contaminants may affect phagocytic processes but this has not been investigated in much detail. Measurement of phagocytic capability or parameters regulating pathogen entry into the organism has been less detailed. In this study we report on the development of a phagocytic assay using the RTgill-W1 cell line as a model system and of parameters regulating latex bead uptake by these cells. RTgill-W1 is a cell line derived from rainbow trout (*Oncorhynchus mykiss*) gills and preliminary fluorometric microplate assays indicate increasing uptake by these cells of latex beads with increasing time and concentration of the microspheres. Trypan blue was used to quench the fluorescence of non-phagocytized microparticles and cytochalasin B, an inhibitor of actin polymerization, was used to demonstrate dependence of actin polymerization in particle uptake. Fluorescence microscopy was used to visualize internalized microspheres and conditions regulating particle uptake, including temperature dependence, will be presented.

Exposure/effects - mechanistic models / Exposition/effets - modèles mécanistes

Session co-chairs/Présidents: S.B. Brown and/et A. Hontela

Progesterone levels in a key species in coastal sediments, the annelid polychaete, *Hediste diversicolor*.

C. Mouneyrac[1], J. Pellerin[2], C. Durou[1,3], C. Amiard-Triquet[3] et P. Rainbow[4]. [1]Centre d'Étude et de Recherche sur les Ecosystèmes Aquatiques, Université catholique de l'Ouest, Angers, France; [2]Institut des Sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, Qc; [3]Institut des Substances et Organismes de la Mer, Université du Nantes, Nantes, France; et [4]The Natural History Museum, London, United Kingdom.

Keywords: progesterone, reproduction, *Nereis diversicolor*, fecundity, coastal sediments, metals

Abstract

It is now known that sex steroid hormones could be used as biomarkers in marine invertebrates. We have recently shown in marine bivalves, delayed sexual maturation, shift in sex ratios and decreased immune competence in response to metal, pesticides and TBT exposure. Since most of contaminants in coastal areas are stored in sediments, it is therefore pertinent to use polychaetes, well known as bioindicators of pollution and for their ecological importance. Recent data from PNETOX show higher levels of PCBs, pesticides and metals in Baie de Seine (France) when compared to Baie de l'Authie, a reference site. The goal of our work was to determine the levels of progesterone in *Hediste diversicolor* and to study its variations according to environmental factors and contamination. Sex ratios were similar to those reported in the literature while different size classes were observed in the two sites, with larger worms in Baie de l'Authie. Progesterone concentrations vary according to the weight of worms but no relation was observed with contamination levels. In conclusion, the use of progesterone as a biomarker could be promising but further studies are needed to understand the role of this hormone in polychaetes. However, our data show clearly that natural factors can modulate progesterone levels as it was shown in other species.

Introduction

The Atlantic European coastal environment is characterized by a huge richness of aquatic ecosystems: rivers, estuaries, coastal marshes and beaches. These aquatic environments are under increasing stress due to urbanisation in coastal areas, intensive agriculture, massive tourism and accidental as well as deliberate pollution. There is therefore a huge need of tools to monitor water quality, particularly in estuaries, well known to be a sink for contaminants, due to the abundance of fine sediments. They are also areas of high productivity, crucial in the life histories of many fish, invertebrates, birds, etc. Animals living in the sediments therefore come into close contact with the pollutants, and may thereby be chronically exposed.

The coastal and estuarine worm *Hediste diversicolor* is a widespread keystone species along the coasts of Europe present from the Baltic Sea to the Black Sea. This worm is a major link in food webs, and of economic importance as bait for fishing in different European countries. Due to its adaptation capacities and as a burrowing species, it lives in close contact with sediments and pollutants and can be used as a pollution indicator by its facility to accumulate contaminants. Effectively, the levels of pollutants in the sediment are almost the time, linearly reflected in the worms (Luoma and Bryan 1982). Moreover, the worm is easily recognized in the field and could be acquired easily. Thus, *H. diversicolor* is a good model organism for investigations on the ecotoxicological impact of multiple organic and metal pollutants in sediments of European coastal areas (Scaps 2000). Among these areas, the choice of Baie de Seine as a contaminated site has been validated by Guezennec et al. (1999) and RNO (2000), and more recently in the case of the Programme national d'écotoxicologie (PNETOX- Ministère de l'Écologie et du Développement Durable) in France. In contrast, recent data from PNETOX have shown that Baie de l'Authie can be considered as a good reference site (Personal communication from Jean-Claude Amiard; jean-claude.amiard@isomer.univ-nantes.fr). A lot of contaminants present in marine environments can exert endocrine disruptions: organochlorines like DDT and metabolites, herbicides, (atrazine, chlordane, dieldrin, lindane), BPCs, dioxins, organotins and natural or synthetic estrogens (Tyler et al. 1998). Some metals can also exert such effects like Cd and Pb (Lee and Noone 1995, Olsson et al. 1995).

Among the various hypotheses underlying the effects of endocrine disruptors on reproduction, inhibition of steroidogenesis is of particular importance. For example, in male and female sea stars, it has been found that the effects of Cd on reproduction were related to decreased levels of progesterone and testosterone. Moreover, an important change in endogenous steroid content has been discovered in the clam *Ruditapes decussata* exposed to TBT (Morcillo and Porte 2000).

Progesterone levels are generally measured in order to estimate the degree of sexual maturation. Literature data is abundant on this subject in different species, namely, fish, molluscs and bivalves. No data is available at this date on a key species in coastal environments, *H. diversicolor* (Polychaete Annelid). The majority of studies on endocrine regulation of reproduction within the Annelid Polychaetes are related to neuroendocrinology aspects. It has been demonstrated that the neurosecretory activity of the cerebral ganglia was correlated with growth and the reproductive cycle (Golding 1987). A highly distinctive pattern of endocrine control of development and reproduction is shown in these worms. These animals are characterized by a monotelic (semelparous) pattern of reproduction in which a single, climatic reproductive event is followed by death. The cerebral neuroendocrine system in these worms promotes growth and regeneration of lost posterior segments, exercises a trophic as well an inhibitory influence on reproduction. Reduction in the rate of hormone secretion in female nereids is known to result, at least in part, from the accumulation of maturing oocytes, an effect mediated by a chemical factor (Porchet and Cardon 1976). Generally, sexual steroids play a critical role in sexual development and are synthesised in steroidogenic tissues from a common precursor, the cholesterol. Therefore, the major aim of this project was initially, to verify the existence of progesterone and secondly, to assess the physiological status of *H. diversicolor* in two paired sites – control vs contaminated – from the Atlantic North West coast of Europe, contrasted by different degrees of pollution.

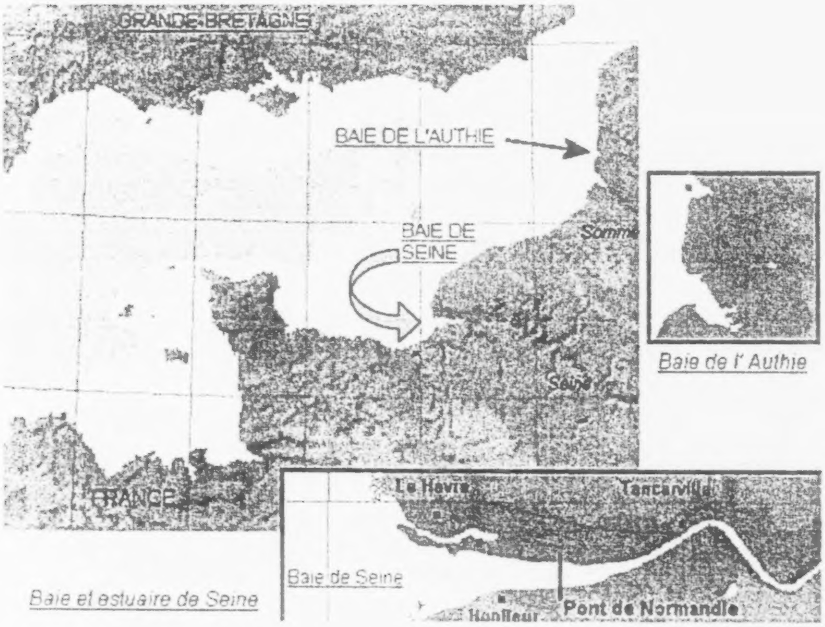


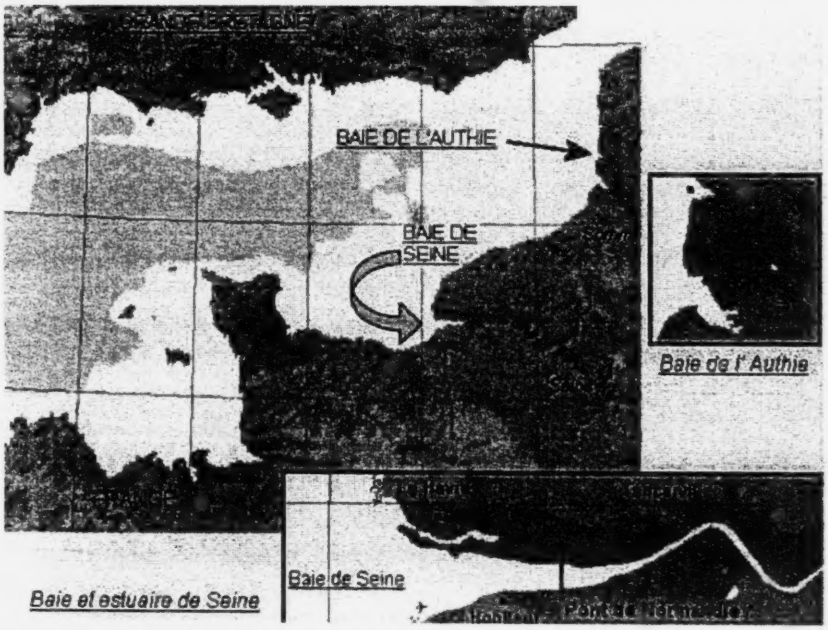
Fig. 1. *Hediste diversicolor* were collected by hand, in February 2003, from littoral sediments (upper 20 cm depth) at Baie de l'Authie (50° 23' 44 ''; 1° 33' 46 ''), the reference site and at Baie de Seine (49° 25'12''; 0° 14' 05'') a contaminated site.

Material and methods

H. diversicolor were collected by hand, in February 2003, from littoral sediments (upper 20 cm depth) at Baie de l'Authie (50°23'44"; 1°33'46"), the reference site and at Baie de Seine (49°25'12"; 0°14'05") a contaminated site (Fig. 1). Worms were rinsed in water collected in situ, placed in cryotubes, quickly frozen in liquid nitrogen and transported from the field to the laboratory. They were stored at -80°C until analysis. In order to estimate the number of eggs and their diameter, 8 worms from each site, have been collected and immediately stored in 70% ethanol until analysis. Therefore, there could be no changes in oocyte size resulting from dilution or concentration of the storage medium. Salinity in Baie de l'Authie was between 25,7 and 28‰. In Baie de Seine, salinity was of 18‰. Temperature in Baie de l'Authie was -1,1°C while it was 4°C in Baie de Seine.

Sex determination has been ascertained by microscopic examination of a sample of coelomic fluid (n=20 for each site). An incision of the worm using a scalpel, after the 11th segment behind the head has been carried out and the content was released on a glass slide, well mixed with a drop of NaCl 0,9% and topped with a cover glass. Determination of number of oocytes and oocyte diameter were estimated using the following procedure. Three segments from each worm (n=8 for each site) were removed from the anterior portion of the worms using a scalpel, approximately twelve segments behind the head. The segments were opened and the contents released. The oocytes were placed on a cavity slide and viewed using a microscope. The dimension measured was maximum diameter of each oocyte to the nearest μm (Batten 1994).

Worm tissue (n=20 for each site) were homogenized in liquid nitrogen by hand with a porcelain mortar and pestle. Then the powder obtained was homogenized with a motorized grinder in 1,5 ml



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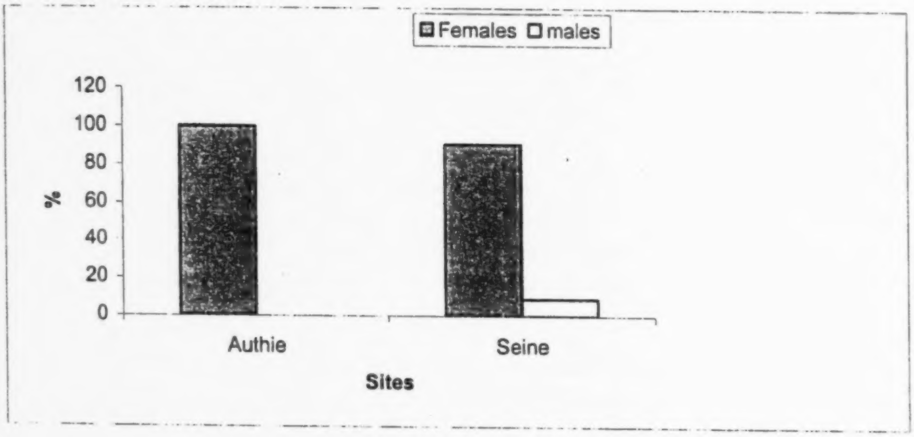

Fig. 2: Sex ratio. Females are predominant in Baie de Seine, with 91 % of the individuals studied. In Baie de l'Authie, the worms observed were 100% females.

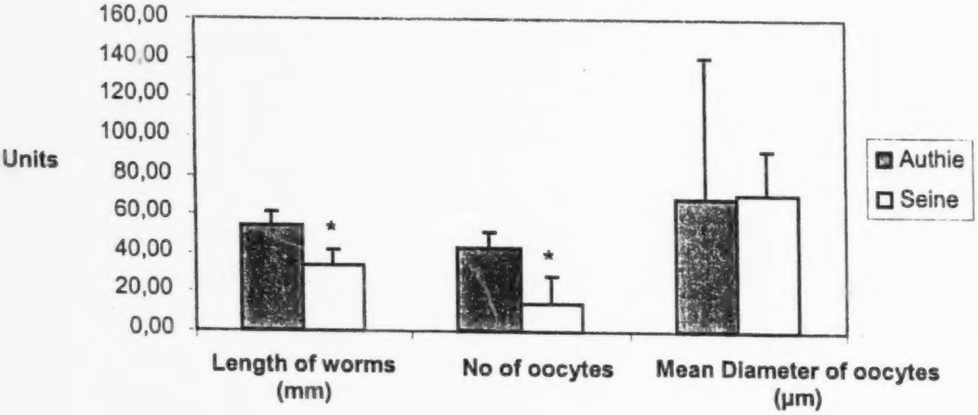

Fig. 3. Sexual maturity indices : length (mm), weight (g w.w.), mean number of oocytes, mean diameter of oocytes (µm).

citrate buffer pH 5,0 for glycogen and lipids. Energy reserves were estimated using the phosphovanillin method (Frings et al. 1972) where lipid determination and glycogen was estimated after enzymatic digestion with amyloglucosidase according to Carr and Neff (1984); olive oil and glycogen from oyster (Sigma, type III) were used as standards for each method respectively.

Progesterone titers (n=20 for each site) were assayed using a commercial ELISA kit (Cayman chemical). Gonad was homogenized in H2O (1:5 w:w) and sonicated 2 times for 30 sec. 400 μL HCl

0.025M were added to 500 µL homogenate for a hydrolysis of 15 min. at 40°C and then 1.25 mL Na2HPO4 were added before organic extraction (Siah et al. 2002). Homogenates were twice extracted with 14 ml dichloromethane and organic extracts were evaporated to dryness under nitrogen at room temperature and redissolved in 250 µl EIA buffer. Progesterone standards were prepared and determinations carried out in duplicate according to the manufacturers instructions. The percentage of steroid recovery in gonad tissue were evaluated by adding known quantities of estradiol and testosterone standards to one part of a homogenate, while the second part contained only endogenous steroids. Percentages of recovery were 94.94 % ± 17.07 (N=8) for progesterone.

The results in this study are presented as the mean of the samples. SPSS® for Windows, version 11.0 was used for statistical analysis. Normality of the distribution has been tested using the Kolmogorov-Smirnoff test. A Student's t-test for a normal distribution was used to compare two groups. To assess multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data with a Tukey's test.

Results and Discussion

Sex ratios indicated females are predominant in Baie de Seine, with 91% of the individuals studied (Fig. 2). In Baie de l'Authie, the worms observed were 100% females. The density of worms has been shown to vary between locations and throughout the reproductive cycle (Chambers and Milne 1975). Sexes are dioecious and worms are semelparous. Sex ratio in favour of females seems common although varying between localities (Clay 1967).

Fig. 3 illustrates different sexual maturity indices: length (mm), weight (g w.w.), mean number of oocytes, mean diameter of oocytes (µm). Worms originating from the Authie site are significantly longer and larger by comparison with those from Baie de Seine. In contrast, no difference has been noted in the number of oocytes. However, for both sites, number of oocytes were quite small, a number of 1000-10000 being an acceptable number to assess the fecundity of *N. diversicolor* (Chambers and Milne 1975).

Length of mature worms usually are found in the range of 60-120 mm (Chambers and Milne 1975). However, in our study (Fig. 3), *N. diversicolor* were smaller (40-60 mm). Length at maturity for females has been reported for lengths near 60-70 mm. Although the worms studied were smaller, we observed that they were sexually mature; the smaller size could be explained however by nutritional conditions in the sites studied.

Weight classes' distribution in worms originating from the two sites and used for progesterone measures were established (Fig. 4). We can observe five distinct weight classes in worms from Baie de l'Authie with predominant organisms which weight was comprised between 100 and 200 mg. However, only 2 weight classes have been distinguished in Baie de Seine with lesser weight (predominance of the =100 mg weight class) of animals collected.

Progesterone concentrations were positively correlated to weight of individuals (Fig. 5). No significant differences have been noted between linear regression slopes. Progesterone levels were significantly different (p=0,005) according to the weight of worms originating from Baie de l'Authie (Fig. 6). It is why the figure illustrates hormonal levels by two distinct weight classes, between 0-200 mg and >200 mg. Given that the number of eggs was significantly different between Authie and Seine sites, the latter one having less eggs, it is possible that contaminants in the Seine site have affected reproduction. When smaller worms are selected, no significant differences (p=0,089) are however observed between sites for the same size classes of worms, since in Baie de Seine, only small worms were available.

Glycogen and lipid energy reserve concentrations in *H. diversicolor* were measured in both sites

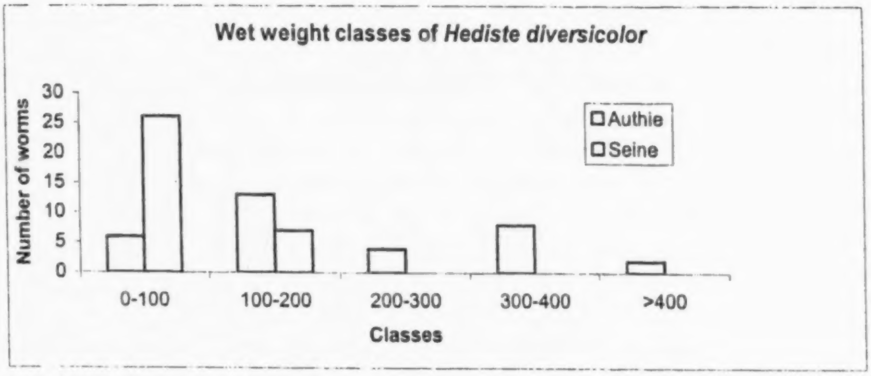

Fig. 4. Weight classes of *Hediste diversicolor* in both sidies.

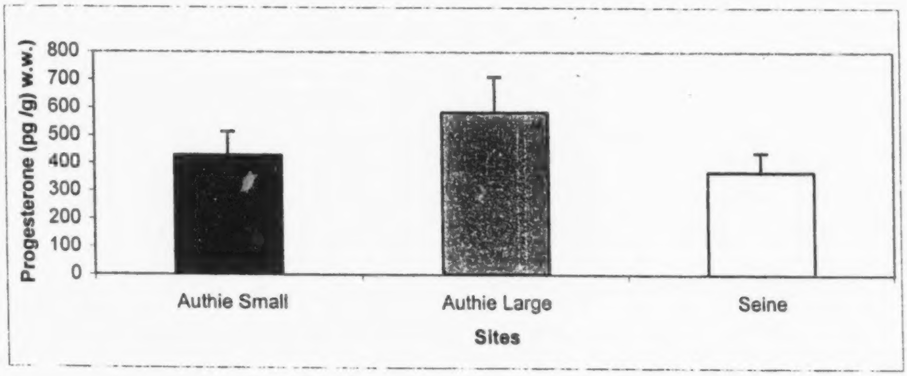

Fig. 5. Progesterone levels in Authie large and small worms, and in Baie de Seine worms

(Data not shown). No significant differences in lipid concentrations determined in worms from both sites (Baie de l'Authie vs Baie de Seine) or weight classes (Baie de l'Authie only available) have been observed in February 2003. However, the seasonal pattern of proteins, glycogen and lipids are different between the two sites. In Baie de Seine, concentrations in energy reserves are smaller which may be due to the lower numbers of eggs found in these worms (Fig. 3).

Conclusions

Comparisons of the different measures carried out in *H. diversicolor* show clearly a direct relationship between weight and progesterone levels. This difference cannot be due to different stages of sexual maturity, since all worms were found mature. However, energy reserves were low in Baie de Seine, reflecting probably the low nutritional quality as well as the levels of contaminants of this site and these factors could also explain the low numbers of eggs.

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Mechanisms of cadmium-induced adrenotoxicity in trout (*Oncorhynchus mykiss*) **adrenocortical cells: involvement of calcium channels.** A. Hontela[1] and A. Lacroix[2]. [1]University of Lethbridge, Lethbridge, AB; and [2]Université du Québec à Montréal, Montréal, Qc.

The mechanisms of action of Cd (Cd2+) on ACTH-stimulated adrenal steroidogenesis of cortisol in rainbow trout, *Oncorhynchus mykiss*, were investigated *in vitro* in dispersed adrenocortical cells. Adrenotoxicity of Cd2+) was increased in absence of extracellular Ca2+, but was prevented in supplemented Ca2+ medium, suggesting that Cd2+) competes with extracellular Ca2+. The implication of Ca channels on stimulated cortisol synthesis was also investigated using BAY K8644 (BAY) and nicardipine as agonist and antagonist, respectively, of voltage-dependent Ca channels. Pretreatment of cells with BAY and exposure to Cd2+) increased the inhibition of ACTH-stimulated secretion but not PREG-stimulated secretion. These results suggest that opening of voltage-dependent Ca channels with BAY may allow Cd2+) entry at the same time as Ca, thus increasing toxicity of Cd2+). Nicardipine also increased the inhibition of ACTH-stimulated secretion by Cd2+), suggesting that voltage-dependent Ca channels may not be the only way of entry into adrenocortical cells. Finally, the influx of Ca2+ and/or Cd2+) was measured in stimulated adrenocortical cells using Fluo-3. The data suggested that Cd2+) influx was significantly enhanced by PREG stimulation. The use of dispersed teleost adrenocortical cells provided new data on the interactions between Cd2+) and Ca2+ in a teleost endocrine cell model. (Funded by NSERC)

Effects of hydroxylated PCBs (OH-PCBs) on thyroid status of rainbow trout (*Oncorhynchus mykiss*). A. Buckman[1], A.T. Fisk[2], K.R. Solomon[3] and S.B. Brown[4]. [1]University of Guelph, Guelph, ON; [2]Warnell School of Forest Resources, University of Georgia, GA; Centre for Toxicology, University of Guelph, Guelph, ON; and [4]Environment Canada, National Water Research Institute, Burlington, ON.

Although hydroxylated PCBs (OH-PCBs) disrupt mammalian thyroid status, the impact of these halogenated phenolic compounds (HPCs) on the thyroid system in fish has not been evaluated. To address this issue, we fed rainbow trout (*Oncorhynchus mykiss*) held at 12°C food spiked with 9 OH-PCB congeners and pentachlorophenol at nominal concentrations of 1 μg/kg and 10 μg/kg (total HPC) for 60 d. These specific OH-PCBs were selected on the basis that they are the predominant HPCs in the blood plasma of Lake Ontario lake trout (*Salvelinus namaycush*). Plasma and tissue hormone analysis were performed using radioimmunoassays for T3 and T4 to quantify changes in hormone levels. Liver thyroid hormone (outer and inner ring) deiodinations were used to investigate thyroid metabolism. Results of this experiment show the potential for OH-PCBs to disrupt thyroid status in fish.

Tracking DDT pathways to tree swallows (*Tachycineta bicolor*) **in Point Pelee National Park.** M. Sebastian[1], J.J.H. Ciborowski[1], J.E.G. Smits[2] and G.R. Bortolotti[3]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; [2]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [3]Department of Biology, University of Saskatchewan, Saskatoon, SK.

We examined the bioaccumulation of organochlorines in tree swallow nestlings from their insect diets in Point Pelee National Park (PPNP). Terrestrial organisms like birds accumulate organic contaminants from their food (biomagnification) only. DDT and other organochlorines are bioaccumulative. DDT was heavily used in PPNP during the 1950s and 1960s. Although its use was banned in the 1970s, there are still high levels of DDT in the park environment. Tree swallows (*Tachycineta bicolor*) were studied for one breeding season (April-August 2002). Food boluses (insects) were collected from swallow nestlings, identified to family, and quantified. We used light trapping and sweeping to collect adult insects for contaminant and stable isotope analysis. Nestling tissues were also assayed for these analyses. The major insect groups in the food of nestlings by biomass were mayflies (Hexagenia; 41%), midges (Chironomidae; 35%), robber flies (Asilidae; 10%) and ants (Formicidae; 7%). Nestlings' contaminant burdens broadly reflected those of their diet items. Swallows consuming aquatic insects (mayflies, midges) had highest tissue levels of PCBs. Nestlings fed terrestrial insects had highest levels of DDT.

Temperature modifications of PAH-induced CYP1A activity in fish. J. Dungavell[1], T. Cross[1], J. Ennis[1] and P.V. Hodson[2]. [1]School of Environmental Studies, Queen's University, Kingston, ON; and [2]Department of Biology, Queen's University, Kingston, ON.

Temperature is a known modifier of the uptake, metabolism, and excretion rates of polycyclic aromatic hydrocarbons (PAHs) in fish. Therefore, temperature is of great importance in ecological risk assessment (ERA) of PAH because alterations to the metabolism of PAHs will have effects on the contaminant's toxicity to the organism. The effects of acclimation and exposure temperature were assessed in rainbow trout (*Oncorhynchus mykiss*) exposed to β-napthoflavone (BNF), a known CYP1A inducer, and 7-isopropyl-1-methylphenanthrene (retene), an alkyl-substituted PAH. In lab experiments with fish exposed to BNF, both acclimation and exposure temperature caused marked changes in CYP1A response to PAH exposure. In particular, CYP1A induction, and presumably metabolic rate, were lower at very high temperatures. This suggests that fish have lower metabolic rates of PAH at high temperatures, and that CYP1A requires a longer period than the bioassay duration to acclimate to a new temperature. Also, decreases in CYP1A induction were noted as exposure temperatures deviated from the optimum water temperature preferred by trout, demonstrating that temperature stress causes reduction in CYP1A induction. Experiments with retene found similar results and thus, metabolism of PAHs is affected in a similar manner to BNF under acute temperature change. Temperature has been shown to modify the metabolism of PAHs by fish, and therefore must be accounted for when comparing studies, when interpreting biomonitoring data, and when conducting ERAs.

Thermal modulation of the excretion of benzo[*a*]pyrene in teleosts. B.D. Johnston[1] and C.J. Kennedy[2]. School of Biological Sciences, University of Aberdeen, Aberdeen, Scotland; and [2]Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

The effect of temperature on the biliary excretion of benzo[*a*]pyrene (BaP) in teleosts was studied in live rainbow trout,*Oncorhynchus mykiss*, as well as isolated hepatocytes obtained from rainbow trout, sablefish, *Anoplopoma fimbria*, black rockfish, *Sebastes melanops*, and chub mackerel, *Scomber japonicus*. BaP concentration in blood of rainbow trout declined biphasically with time. With acute temperature increase (8-18°C), clearance rates increased (Qb=0.10 ml/min) and terminal half-lives decreased (X1=2.35 min and X2=3786.2 min). Conversely, with acute temperature decrease (18-8°C), clearance rates decreased (Qb=0.05) and terminal half-lives increased (X1=3.43 min, X2=5250.7 min). The highest BaP body burdens were found in liver, visceral fat, kidney, and gill respectively. Metabolites of BaP excreted to the bile, included diols and tetrols (including 9,10-diol and 7,8-diol), quinones including (3,6-dione), and phenols (including 9-OH and 3-OH). Phase II metabolite excretion from isolated rainbow trout hepatocytes in suspension increased significantly in cells acclimated to 18°C and exposed to BaP at 8°C. Phase II metabolite excretion from trout hepatocytes grown as monolayer cultures did not change with acclimation temperature but was altered concomitantly with acute temperature. Significantly different temperature effects were seen in BaP toxicokinetics in hepatocytes of marine teleosts. BaP excretion is compensated for with respect to acclimatory changes in temperature but responds concomitantly to acute temperature change.

Chronic toxicity of hydrophobic compounds measured using partition controlled delivery. D.Turcotte[1], P. Akhtar[2], Y. Kiparissis[1], P.V. Hodson[3] and R.S. Brown[2]. [1]School of Environmental Studies, Queen's University, Kingston, ON; [2]Department of Chemistry, Queen's University, Kingston, ON; and [3]Department of Biology, Queen's University, Kingston, ON.

In conventional static or semi-static embryo toxicity assays with fish, the nominal concentrations of hydrophobic chemicals are often used to establish the toxic thresholds, which often far exceed the solubility limits of test compounds. We have developed a partition controlled delivery (PCD) method that maintains the concentrations of hydrophobic chemicals in test solutions at or below solubility limits for extended exposure times. Polydimethylsiloxane (PDMS) films containing various concentrations of C1-C4 phenanthrenes (PHE) are deposited on the side of 20 ml vials and equilibrated with test media. Fertilized Japanese medaka *(Oryzias latipes)* eggs are added for a 17 d embryo toxicity test at 26°C. Previous experiments showed the efficacy of the PCD method in comparison with semi-static (24 h renewal) embryo-larval toxicity tests. Current results for the series of PHE indicate that for more soluble compounds (e.g., C1-PHE) the two methods give similar effect concentrations, but for less soluble compounds (e.g., C4-PHE) nominal semi-static effect concentrations are above solubility whereas the PCD method effect concentrations are below solubility. These results show that the PCD exposure method is a more sensitive and more realistic method for assessing embryotoxicity of non-polar compounds. Future work will include study of C1-C4 anthracenes using PCD.

Significance of oil droplets in chemically enhanced water-accommodated fraction. S. Ramachandran[1], P.V. Hodson[2] and K. Lee[3]. [1]School of Environmental Studies, Queen's University, Kingston, ON; [2]Department of Biology, Queens University, Kingston, ON; and [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.
Chemical dispersion is an oil spill contingency measure fraught with controversy due to reports of negative impacts on aquatic life from the dispersant as well as the dispersed oil. While breaking up the spill to reduce shoreline impacts, the dispersant drives the oil into the water column in the form of droplets, hence temporarily increasing the hydrocarbon concentrations. Exposure experiments with rainbow trout using water-accommodated fractions (WAF) showed accumulation of polycyclic aromatic hydrocarbons (PAHs) was 6-1100 fold higher in chemically (Corexit® 9500) dispersed water-accommodated fractions (CEWAF) than WAF. Dispersing crude oils would thus sustain hydrocarbon concentrations in a larger volume of water than if it were not dispersed.The effect of dispersants was due primarily to an increase in the concentration of suspended oil droplets. To examine the role of these droplets in (i) increasing the partitioning of PAHs into solution; or (ii) by adhering to gill filaments thereby facilitating direct uptake, exposure experiments have been conducted using CEWAF from Mesa and Scotian Light Crude Oil with and without oil droplets. Examination of gills from exposed fish showed fluorescing oil droplets adhering to the filaments.

Chemical profile of crude oils in support of toxicity studies. P. Akhtar and R.S. Brown. Department of Chemistry, Queen's University, Kingston, ON.
Several studies have shown that acute and chronic exposure of fish to oil spills can cause toxic effects at all life stages. Most (80%) PAH in oils are alkylated PAH compounds, and a specialized analysis methods must be developed to determine the alkylPAH "fingerprint" of oils and oil fractions. This information will be useful in oil spill emergencies, in preventing exposure of fish populations to the most toxic of oils at critical points in their life cycle, when deciding to use various control, clean-up and remediation options. Recent work on alkylphenanthrene compounds demonstrates that this class of PAH, in contrast to unsubstituted phenanthrene, causes embryo-larval toxicity. We focused on characterizing alkylphenantres fraction of crude oil for their bioavailability. With HPLC a linear correlation between capacity factor and LogP values was developed. It was also found that a better linear correlation exists between capacity factor and alkyl carbon numbers. We calculated the Log P values and number of alkyl carbons for the alkylphenanthrenes in Mesa crude and ANS crude oils. Log P values for alkylphenanthrens in Mesa ranged from 4.87-5.77 and corresponding number of alkyl carbons are 1 to 3. Alkyl-PAH "fingerprint" along with LogP values of components will enable us to predict ecological risk of oil spills in aquatic environments.

The effects of cadmium on the cytoskeleton of the human hepatocellular carcinoma (HepG2) cell line. E. Diringer, E. Welnhofer and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

Cadmium is a well-known environmental and human health hazard, but its mechanism of toxicity at the cellular level is unknown. Age of cells and duration of exposure effects of Cd on the cytoskeleton was assessed morphologically. No significant changes in F-actin and microtubule-associated fluorescent intensities occurred, indicating cytoskeletal components had not depolymerized. However changes in cytoskeletal organization were observed and did show age and duration of exposure effects. Both passage 4 (young) and 6 (older) Cd-exposed cells at 2 and 8 h showed an increased frequency of thick F-actin bands near the perimeter of the cell. Cd-exposed young cells showed stress fibers at 2 and 8 h, while none were observed at either time in older cells. Numerous microspikes were observed in Cd-exposed young cells at 8 h, while none were observed in older cells at 8 h. F-actin cables increased in frequency in older cells at 2 and 8 h, but only at 2 h in young cells. All Cd-exposed cells showed a shift from a radial to a parallel arrangement in microtubules with respect to the nucleus. These changes in cytoskeletal organization may cause decreases in cellular adherence and alterations in cell shape, which have been associated with altered cell function and may lead to toxicity. Age effects may explain some of the variability often seen in cellular toxicity studies. (E.D. funded through a HHMI Undergraduate Biological Sciences Education grant to Canisius).

Developing rapid toxicity tests with the ciliated protozoan, *Tetrahymena thermophila*, **that utilize microwell filter plates**. V.R. Dayeh[1], D. Sotornik[1], D. Lynn[2], N.C. Bols[1] and L.E.J. Lee[3]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Zoology, University of Guelph, Guelph, ON; and [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

As a test organism for evaluating effluent toxicity, *Tetrahymena thermophila* has several advantages over test organisms, such as rainbow trout, and even over animal cell culture alternatives, such as cell lines. In comparison to fish, protozoan tests are more rapid, ethical, and less expensive. Costs are less partially because tests are done on small samples, reducing the expense of shipping large effluent volumes to a central testing facility. Unlike animal cell cultures, tests with protozoan can be done directly in effluent, without adjusting osmolarity or removing bacteria by filtration. This reduces labor and the potential of removing toxicants. These attributes of protozoan could be more fully realized if multiple cultures could be simultaneously exposed to toxicants, quickly washed to terminate toxicant exposure, and then conveniently evaluated for changes in cellular functions. Therefore, *T. thermophila* was exposed to a model toxicant, Cu, in either microcentrifuge tubes or 96-well filter plates and evaluated for changes in energy metabolism, membrane integrity, lysosomal function and phagocytosis. These functional assays used fluorescent dyes and were read rapidly in a fluorescent multiwell plate reader. Exposing *T. thermophila* to Cu in 96–well filter plates and monitoring energy metabolism with alamar Blue provided a superior toxicity test.

Biotransformation of perfluorooctane sulfonamide and ethyl perfluorooctane sulfonamide by rainbow trout (*Onchorhynchus mykiss*) **liver microsomes.** V.P. Palace[1], G. Tomy[1], S. Tittlemeir[2], E. Braekevelt[1], W. Budakowski[1], B. Lau[2], J.G. Eales[3] and J. Plohman[3]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Health Canada; and [3]Department of Zoology, University of Manitoba, Winnipeg, MB.

Rainbow trout (*Onchorhynchus mykiss*) liver microsomes were incubated with n-ethyl perfluorooctanesulfonamide [N-EtPFOSA, C8F17SO2NH(C2H5)], to examine the possibility of *in vitro* biotransformation to perfluorooctane sulfonate (PFOS, $\mathrm{C8F17SO3}^{-}$) and perfluorooctanoic acid (PFOA, $\mathrm{C7F15COO}^{-}$). Incubations were performed by exposing trout liver microsomes to N-EtPFOSA at 8°C in the dark. Microsomes were taken after incubation periods of 0, 2, 4, 8, 16 and 30 h, quenched and analyzed for N-EtPFOSA, PFOS, PFOA and perfluorooctane sulfonamide

(PFOSA, C8F17SO2NH2), a suspected intermediate. Only PFOS and PFOSA were detected in the samples. Two possible reaction pathways are proposed for the conversion of N-EtPFOSA to PFOS: (i) direct conversion of N-EtPFOSA to PFOS by deamination followed by oxidation of the sulfone group, and (ii) deethylation and reduction of N-EtPFOSA to PFOSA, followed by loss of NH2 from this intermediate to form PFOS. The kinetics of formation of PFOS obeyed a zero and first order reaction equally well. The rate of formation (kf) of PFOS for the zero and first order reaction were 0.029 ng/mL/h and 0.015 ng/mL/h, respectively. These findings represent the first report indicating a possible biotransformation of a perfluorosulfonamide to PFOS in fish and may help to explain the detection of PFOS in biota from remote regions.

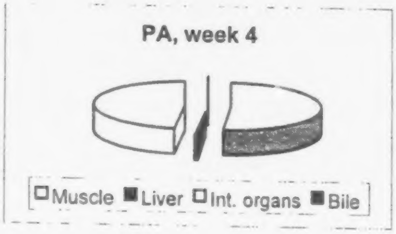
Thyroid and vitamin A status in mink (*Mustela vison*) **fed PCB-contaminated carp** (*Cyprinus carpio*) **from Saginaw Bay**. V.P. Palace[1], P. Martin[2], S. Bursian[3] and G. Mayne. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Environment Canada, Canadian Wildlife Service; [3]Michigan State University, Ann Arbor, MI.
Female mink (*Mustela vison*) were fed diets containing 0, 10, 20 and 30% PCB contaminated carp (*Cyprinus carpio*) from Saginaw Bay, Michigan, throughout breeding, gestation and lactation; weaned juveniles were exposed till 28 weeks old. Plasma thyroid hormones, gland activity and structure, and vitamin A content were measured in 6-week-old kits and juveniles. Total hepatic TEQs of juveniles were 8.80, 27.48, 74.10 and 105.50 ng/kg. Plasma total T4 (TT4) and free T4 (FT4) concentrations in female and male kits fed the 10% carp diet were higher than controls, while kits fed the 20% and 30% carp diet showed non-significant decreases. Plasma TT3 decreased in juveniles with increasing exposure, but only the group fed the 30% diet were significantly different from controls. Overt thyroid toxicity was not apparent as thyroid weight, activity, or histological structure in either kits or juveniles. Significant differences in vitamin A were found in mink fed contaminated carp relative to control mink. Kits fed the 30% carp diet had half the plasma retinol content of controls. Plasma retinyl stearate in kits, and plasma retinyl palmitate in juveniles were reduced in the highest dose group. Hepatic retinol/retinyl palmitate ratio was two times higher in kits and juveniles relative to control mink. Significant reductions in kidney retinol and esters were observed in kits and juveniles fed the 30% carp diet relative to controls. Compensatory mechanisms appear to have prevented significant reductions in circulatory thyroid hormones. Consumption of contaminated carp can induce perturbations in plasma thyroid hormone homeostasis as well as vitamin A status in young mink.

Fate of PAHs in internal organs, muscle and gall bladder bile of finfish. J.D. Leonard and J. Hellou. Department of Fisheries and Oceans, Bedfrod Institute of Oceanograpaphy, Dartmouth, NS.

Polycyclic aromatic hydrocarbons (PAHs) are a class of widely and continuously produced organic compounds that can persist long enough to represent a toxic risk to a variety of organisms (Nisbet and Lagoy 1992, Delistraty 1997, van der Oost et al. 2003). Bioaccumulation and biotransformation of these chemicals have been shown to occur in both vertebrates and invertebrates (Stromberg et al. 1999, Eikenhoff et al. 2003). The aim of this study was to compare the fate, in terms of tissue distribution, of parents and metabolites of three abundant PAHs which were fed to speckled trout (*Salvelinus fontinalis*). This experiment represents a follow-up to our previous observations on the bioaccumulation and biotransformation of PAHs in field and laboratory finfish (e.g., Hellou and Warren 1997, Hellou et al. 1999, 2002, 2003; Leonard and Hellou 2001).

The question pursued presently relates to the long-term fate of PAHs in finfish exposed through the diet. For 70 d, the trout, maintained in tanks at 10°C were fed pellets spiked with phenanthrene (PA), fluoranthene (FL), and pyrene (PY) to yield a dose of each PAH of 0.33-0.38 mg/kg fish per day. Subsequent to this, the trout were fed non-spiked food for 28 d. Over the full length of the experiment, the fish grew well and showed no obvious effects from the PAH. Every 14 d, 5 randomly selected fish were sacrificed and dissected with gall bladders, livers, other internal organs and carcasses frozen immediately. These tissues were subsequently extracted and analyzed by HPLC for the parent PAHs and for metabolic products such as glucuronides and phenols.

The concentration of parent compounds in both the internal organs and the muscle were always of PA > FL > PY during the period of exposure. In the livers, only PA was above detection level. Levels were always higher in the internal organs than in the muscle, with the ratio of concentration in internal organ to muscle increasing with time, for each of the three compounds. The total tissue burden of PA in muscle showed a steady increase. In the internal organs, levels of the three parents increased over time, with FL approaching a maximum equivalent to one day's dose and PY significantly less. PA showed a much more precipitous increase, particularly after day 42 reaching a maximum higher than the equivalent of five day's feeding. On day 84, after 14 d with no PAHs in the feed, PA in the internal organs was by far the slowest to disappear, as approximately 40% of the maximum concentration was still present. Even two weeks later at day 98, a significant level of PA was fstill detected.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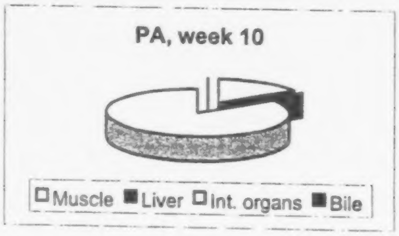
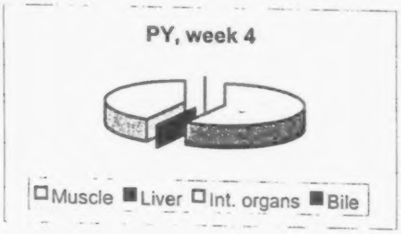
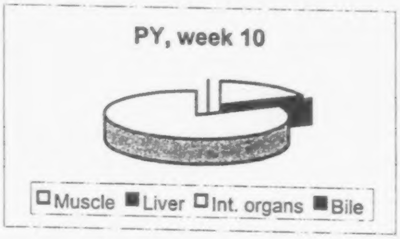
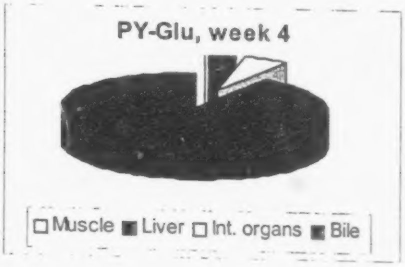
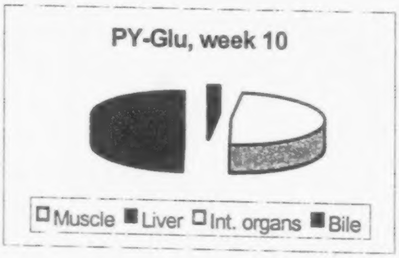
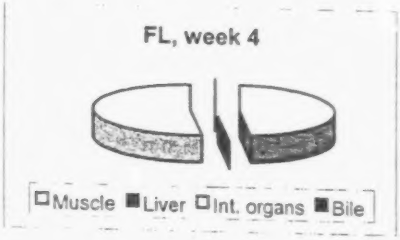

PA, week 10

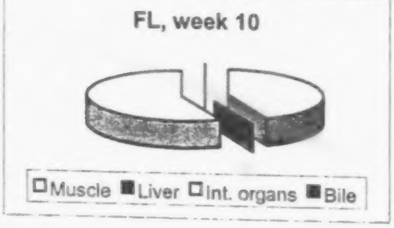

PY, week 4


PY, week 10


PY-Glu, week 4


PY-Glu, week 10


FL, week 4


FL, week 10

For PA and FL, no phase I or phase II metabolites could be detected, even in the bile. For PY, the glucuronide was observed in the gall bladder with levels generally higher than those observed for the parent compound in any whole compartment. Its highest levels corresponded to 15% of the daily dose. The PY glucuronide was also observed in the internal organs and livers at lower levels. Levels of PY glucuronide dropped rapidly once the fish were given uncontaminated feed and were less than 5% of the maximum by day 84. The complete absence of FL and PA metabolites, even in the bile, was in sharp contrast to an earlier single dose short term studies of flounder where levels of the PA glucuronide and FL-dihydrodiol were comparable to PY derivatives (Hellou and Leonard, 2003).

Understanding the distribution of PAHs and their metabolites in tissues of finfish exposed in the laboratory will help in interpreting the source and duration of exposure in field collected finfish. Combining investigations of chemical fate and biological effects will also help in risk assessment (Fiejtel et al. 1997). As well, trout are a good model organism for other vertebrates.

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Risk assessment and the magic duck. G. Wickstrom and M. Cameron. Keystone Environmental Ltd., Burnaby, BC.
The selection of measurement receptors can significantly affect the results an ecological risk assessment. The exposure factors applied to each receptor, such as diet, home range size, body weight, levels of contamination in food and exposure media, and the duration of exposure also significantly affect the results. In an attempt to make use of readily available information and/or in an effort to produce a "conservative" receptor, some risk assessments appear to incorporate questionable exposure factors. These appear to be the exposure factors of two or more similar species. In one notable case we refer to as the "magic duck," a mallard was modelled as diving 20-30 feet to feed solely on benthic invertebrates. Certainly these fictitious exposure factors result in a conservative estimate of exposure to mallards where contamination is sediment related. However, the result is an unrealistic assessment of a species that does not exist. We propose that the time spent creating these fictitious receptors or exposure factors would be better spent reducing the uncertainty associated with assessment of "real" species that are representative of actual receptors of concern. In addition, the use of fictitious receptors may actually result in the under estimation of potential risk based on inappropriate toxicity reference values, and/or the lack of considering appropriate receptors.

Toxicity of naphthenic acids in tissue culture water and environmental water to fish cell lines. V.R. Dayeh[1], L.E.J. Lee[2], D.G. Dixon[1] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.
Naphthenic acids (NAs) are complex mixtures of saturated carboxylic acids found at high levels in oil-sands tailings. The exact chemical composition of NAs is not known and their effects on biota have been poorly investigated. Reclamation of oil from the oil-sands in Alberta has led to environmental concerns regarding its extraction procedures as large volumes of tailings are released into the environment. The present study was designed to investigate the cytotoxic effects of crude NAs prepared from a settling basin and from commercially purchased NAs to cell lines from rainbow trout (*Onchorhynchus mykiss*) in tissue culture water and environmental water samples. Rainbow trout cell lines derived from gill, liver, and spleen were examined for impairment in several cellular functions using a fluorescence microplate reader. Cell membrane integrity, mitochondrial activity and lysosomal function were monitored at various NA concentrations. These bioassays could potentially be used to examine for the presence of naphthenic acids in environmental water samples.

The toxicity of photomodified polycyclic aromatic hydrocarbons to Japanese medaka *(Oryzias latipes)* embryos. A.J. Farwell, M. Croft, S.M. Rhodes and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.
Alkylated polycyclic aromatic hydrocarbons (alkyl-PAHs) are naturally occurring compounds found in process-affected water following the extraction of bitumen mined from the Athabasca oil sands in northern Alberta, Canada. Previous studies have shown that the base-neutral hydrocarbon fraction of process-affected water (rich in alkyl-PAHs) significantly increased the frequency and severity of blue sac disease (BSD) symptoms, while reducing larval fork length at hatch in Japanese medaka (*Oryzias latipes*) 18 d early-life stage bioassays. The C2-substituted dibenzothiophenes (DBTs) were found to be the most common hydrocarbons of the parent and methylated USEPA PAH priority pollutants (16) measured in the extract. This extract was exposed to simulated solar radiation (SSR) to determine the effects of photomodification on the toxicity of a complex PAH extract. The Japanese medaka 18 d early-life stage bioassay protocol was modified from previous studies in order to accommodate for the photomodification, and the results were found to be similar for the measured endpoints (hatch success, hatch length, time to hatch and evidence of BSD). The extract, exposed to simulated solar

radiation (SSR) for 1 and 4 d, showed decreased toxicity to Japanese medaka embryos relative to the non-photomodified extract. With 4 d SSR exposure, there were no significant changes in hatch length or BSD symptoms whereas there was slight increase in time to hatch.

Use of QSARs in the categorization of discrete organic substances on Canada's Domestic Substances List. P. Robinson. Environment Canada, Gatineau, QC.
One of the new initiatives in the *Canadian Environmental Protection Act*, 1999 (CEPA 1999) requires the Minister of the Environment and the Minister of Health to "categorize" (Section 73, CEPA 1999) the approximately 23 000 substances on the Domestic Substances List (DSL) prior to September 14, 2006. Environment Canada is responsible for categorizing the substances on the DSL as being persistent or bioaccumulative and inherently toxic to non-human organisms. If the substance is determined to meet the criteria, then a Screening Assessment is to be prepared. In order to categorize the substances on the DSL within the mandated time frame, and prepare screening assessments as necessary, it is recognized that QSARs will be relied upon to fill data gaps in many cases. The substances on the DSL are composed of many classes of organic and inorganic chemical compounds, as well as polymers, and complex substances of variable composition (UVCBs). QSARs will be used to generate the necessary data for a large number of compounds; however, for many of these substances appropriate QSARs are not available. Consequently, QSAR generated data is only to be used when it is believed to be a valid representation of a substance's physical/chemical or toxicological characteristics. Environment Canada's mandate to categorize the substances on the DSL by the Existing Substances Branch will be outlined, as well as the application and issues related to the use of QSARs in this process.

Experimental sediment exchanges between natural and constructed wetlands on oil sands leases, near Fort McMurray, Alberta: a look at the relationship between sediment treatments and benthic macroinvertebrates colonizers. L. Barr[1], J.J.H. Ciborowski[1], N. Cooper[2] and L. Foote[2]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON: and [2]Department of Renewable Resources, University of Alberta, Edmonton, AB.
Mining for oilsands has been taking place around the Fort McMurray area since the 1960s and is anticipated to exceed 1406 sq km in magnitude by the year. The subsequent reclamation of these areas is of utmost importance due both to the magnitude of the area being affected and the seriousness of the effect put on the original ecosystems by the mining process. The sequestering of mining by-products is a topic of considerable concern for the mining companies involved due to the large quantities of material produced. This reciprocal transplant study looks at the suitability of the mining by-product consolidated tailings as sediment for constructed wetlands. The reciprocal study enables the isolation of the effect from tailings associated water from the effect of consolidated tailings sediment on benthic macroinvertebrate population health. Sediments from two natural wetlands (opportunistic and reference) are transplanted with sediment from a constructed wetland layered with 4 m of consolidated tailings. Both core and sweep samples are taken to gather adequate sampling of associated benthic species both rare and common. The effects of macrophyte presence in these plots will also be able to be contrasted with the macroinvertebrates sampled there to investigate a possible relationship.

Effects/impacts - ecological effects/Effets, impact - importance écologique

Session co-chairs/Présidents: A. Hontela and/et S.B. Brown

Long-term survival of early life stage fish exposed to PAH-contaminated sediment. M.E. Bowerman, H.E. Tarnowski, C.W. Khan and P.V. Hodson. Department of Biology, Queen's University, Kingston, ON.

Early life stages (ELS) of fish exposed to PAH-contaminated sediment show increased rates of mortality, CYP1A induction, malformations, reduced growth, and subcutaneous edema in the yolk and pericardial sacs. Malformations and reduced growth incurred during a fish's early development may hinder their future competitive ability. Rainbow trout (*Onchorhynchus mykiss*) were exposed from eyed egg to swim-up both *in situ* and *ex situ* to Kingston Harbour sediment, an area contaminated by deposits of coal tar. Each fish was measured for length, scored for blue sac disease symptoms and then transferred to clean water in holding facilities for 6 months of growth and development. A sub-sample of the exposed larvae was preserved for immunohistochemical (IHC) localization of CYP 1A protein, to characterize PAH exposure. The results of these experiments provide an estimate of the delayed effects of exposure to PAH contaminated sediments during larval development and of the long-term consequences of short-term responses to PAH.

Comparisons of metallothionein concentrations, cadmium accumulation and Gene expression of metallothionein 20IIa cDNA in gills of *Mytilus edulis* after cadmium and estradiol Exposure. J. Pellerin[1], J.-C. Amiard[2] and C. Gratas[3]. [1]Institut des Sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, Qc; [2]Institut des Substances et Organismes de la Mer, Université de Nantes, Nantes, France; et [3]Institut de Biologie, Unité Inserm 419, Nantes, France.

Abstract

Metallothioneins (MTs) can be induced in many species by Cd, Zn, Cu and by estrogens. There is therefore a need to understand responses of MTs since it is recognized as a biomarker of exposure to metals. The goal of this study was to verify if estrogens could induce MT synthesis and gene expression of MTIIa cDNA in the blue mussel(*Mytilus edulis*). In a 14 d *in vivo* exposure to 50 μg/L Cd in the presence or absence of 4 mg/L estradiol (E2), comparisons were carried out between bioaccumulation of Cd, MT concentrations measured by polarography and MT IIa cDNA gene expression. Cd bioaccumulation in gills of Cd and Cd + Estradiol groups was observed in both soluble and insoluble fractions while Cd was found mostly in insoluble fractions in control groups. MTs showed a high correlation with total Cd (R2 = 0,8297). MT IIa gene expression was significantly increased by Cd and Cd+E2 but not by estrogens alone, when compared to either control or E2 groups. MT IIa gene expression was well correlated to MTs measures. We can then conclude that functional genomic in *M. edulis* could be used as sensitive tools to assess gene dysfunctions and exposure at least to metals.

Introduction

Metallothioneins (MTs) are non enzymatic proteins with a low molecular weight, a high cystein content, no aromatic amino acids and are characterized by their heat-stability. Multiple isoforms have been identified and polymorphism appears to be particularly important in invertebrate species when compared to mammals. Physiological responses to pollutants may induce changes in gene expression as part of an organism's homeostatic mechanisms. Thus, molecular genetic biomarkers have the potential to characterize more precisely these different forms of MTs, permitting to improve our knowledge of their peculiar biological roles (Lemoine et al. 2000, Syring et al. 2000, Tanguy et al.

2001, Tanguy and Moraga 2001). Contamination *in situ* implies interactions between pollutants and it is possible that molecules induced by heavy metals, per exemple like MTs, could attenuate the deleterious effect of another substance. It is now well recognised that target mechanisms of pollutants are numerous and often shared by many substances present in the environment. However, among the interactions between contaminants, Reis-Henriques and Coimbra (1990) have postulated that estrogens in *Mytilus edulis*, could induce MTs synthesis, a mechanism facilitating therefore bioaccumulation of Cd and Cu. Olsson et al. (1995) have for their part, shown that in fish, non MT-protein synthesis was induced and binding of Cd with these non-MT proteins has repressed the induction of MT and therefore, increased the toxicity of Cd. The aim of this paper was to verify if the presence of estrogens in Cd-contaminated marine ecosystems could be beneficial to blue mussels (*M. edulis)*. We then verified in this paper, the response of an isoform of metallothionein, MT IIa, known to be induced by Cd (Lemoine et al. 2000) and the gene expression of MT, MT IIa cDNA to Cd, in the presence and absence of 17β-estradiol in *M. edulis*.

Material and Methods

Mature blue sea mussels were collected in Baie de Bourgneuf (Atlantic coast of France) in September 2002 from an aquaculture farm. The mussels were acclimated one week in seawater prefiltered and provided by Vendée Naissain (Bouin, France). Thirty mussels were placed in aerated 30 L experimental basins and exposed for 2 weeks to Cd (1,250 μg/kg) and 17β-estradiol (100 μg/kg). Contaminants were injected twice in mussel posterior adductor muscle, at one-week interval. Following exposure, gill tissues were excised under sterile conditions, frozen in liquid nitrogen and stored at -80°C until required. Gills from mussels collected in situ served as the native controls. Experimental controls came from gills of mussels injected with 100 μL NaCl 0,15M and treated in the same manner as the exposed mussels. Exposure was carried out with 17β-estradiol, Cd, and Cd with 17β-estradiol. All treatments were done in duplicate.

Metal and metallothionein were determined using the following procedures. After acid digestion, metals were analyzed by atomic absorption spectrophotometry according to the methods (Amiard et al. 1987) validated by controls of internal quality (standard reference materials of mussel tissues: BCR/278R-N°188) and external quality (Campbell et al. 2000). Metals concentrations were determined in gills after centrifugation both in the soluble fraction, where MTs are measured, and in the particulate fraction, the sum of these fractions is considered as the total metal concentration in this organ.

RNA was extracted according to the classic guanidine thiocyanate / phenol / chloroform method; AGPC extraction (Chomczynski and Sacchi 1987), using RNAwiz™ from Ambion. Treatment of RNA extracts with DNase was carried out to remove any DNA present. Reverse transcription was carried out from 2,5 μg RNA, using SuperScript II RT and oligo(dT)12–18 primers (Gibco-BRL, Paisley, UK), according to manufacturer's instructions.

PCR was performed using the Brilliant™ SYBR Green QPCR Master Mix (Stratagene U.S.A.) : in a 25 μL reaction volume, using 1 μL of RT-reaction, 1.75 μL MgCl2 (Promega, Southhampton, UK), 2.5 μL of buffer, 1 μL of dNTP (Promega), 2 μL of primers TIT20I(S) and TIT20T(AS), 0.5U of Taq DNA polymerase (Promega) and the buffer supplied with the enzyme. PCR amplification was carried out using touchdown PCR, with 25 cycles of 30 sec. at 95°C, 45 s at 60°C, 30 sec. at 72°C, 1 minute at 95°C and followed by an additional 20 cycles at a temperature of 60°C. Primer sequences were derived from *M. edulis* Mt IIa as described in Barsyte et al. (1999) and for actin as an housekeeping gene, in Luedeking and Koehler (2002). Primers were supplied by Sigma-Genosys (Table 1).

Calculations were done using the following steps for each duplicate:
CT (gene expressed in controls) – CT (housekeeping gene) = Δ Ct (calibrator);
Δ Ct (sample) – (Δ Ct calibrator) = $\Delta\Delta$ Ct;
Calcualtion of fold increase between Controls and Cd±E2 = $2^{-\Delta\Delta Ct}$.

Table 1. Primer sequences of MTIIa and actin

	Protein ID	Primer Sequences
Metallothionein IIa	CAA06553.1	TIT20I(S) 5'ATTGCATCGAAACAAACGTG TIT20T(AS) 5'-TTTGCAGTTTGTTGGACCAG
Actin	AAD48064.1	ACTINAY(S) 5' AGCCACATACGTAGCCATCC ACTINAY(AS) 5'-TCGGTTAAGTCTCGTCCAGC

Results

A relationship was established in Fig. 1, between Cd and MT concentrations in gills of *M. edulis* L. Native mussels sampled in a farm site have showed low levels of Cd and MTs before being exposed to the contaminants. Control mussels who were exposed for two weeks to filtered water from an aquaculture site, accumulated Cd to a concentration of 41,95±15,74 ng/g w.w. A similar pattern and similar values for Cd bioaccumulation were observed for mussels exposed to 17β-estradiol. MTs were also induced similarly in control and E2 exposed mussels and proportionally to Cd accumulation but with levels lower than mussels exposed to Cd and to Cd+E2. A sharp increase in MT and Cd concentrations was observed in these two groups but no differences could be observed when E2 was combined to Cd. It can be seen in Fig. 2, a significant relationship between MT and Cd concentrations for all groups with a square regression coefficient of 0,8297.

Native control mussels in Fig. 3, show a different partitioning of Cd between insoluble (60%) and soluble (40%) fractions. The experimental control mussels show, for their part, a shift in this partitioning, with 32% of Cd in the insoluble fraction and 68% in the soluble one. The same trend was observed in E2-exposed mussels (27% and 73%), in Cd-exposed mussels (22% and 78%) and Cd+E2-exposed mussels (19% and 81%). This shift could be well observed in Fig. 4, where the significant relationship between total Cd (in both fractions, S1 and P1) and Cd in S1 and P1 show different slopes reflecting the increase of Cd in the soluble fraction as total Cd concentrations increase. Mt20 IIa gene expression (Fig. 5) has followed the same pattern as MT protein synthesis with a slight but not significant induction in control and E2-exposed mussels. A sharp and statistically significant increase in gene expression is observed for either Cd and Cd+E2 exposed mussels.

Discussion

For the first time to our knowledge, data presented here, show the direct relationship between gene expression of metallothionein MT IIa cDNA, measures of the protein by polarography and Cd bioaccumulation, in response to Cd and estradiol exposure. These results are well in accordance from those of Lemoine et al. (2000) which showed high levels of Mt 20 mRNAs after Cd exposure.

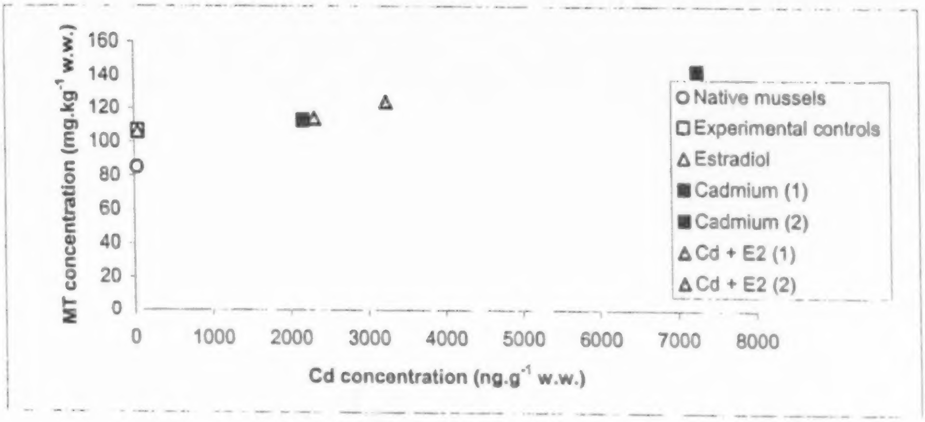

Fig. 1. Relationship between total cadmium concentrations and MT concentrations in gills of *Mytilus edulis*. Blue mussels were exposed for 2 weeks to Cd (1,250 μg/kg) and 17β-estradiol (100 μg/kg). Contaminants in a 100 μL volume of NaCl 0,15M were injected in the posterior adductor muscle. Responses were compared between native mussels (○) sampled in an aquaculture plant in Baie de Bourgneuf (France), mussels exposed to NaCl and seawater from the same area (□) experimental controls), mussels exposed to E2 (△), Cd (■) and Cd + E2 (▲). Cd and Cd + E2 groups are represented twice to show differences between duplicates.

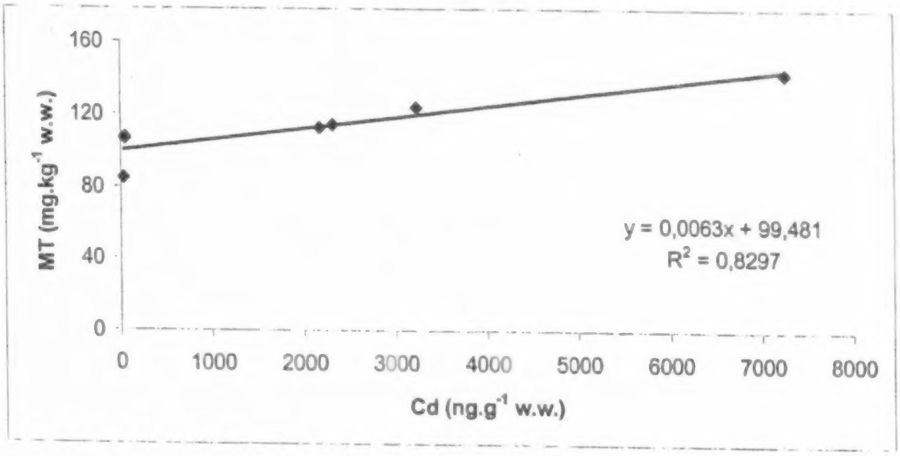

Fig. 2. Statistical relationship between total cadmium concentrations and MT concentrations in gills of *Mytilus edulis*. Blue mussels were exposed for 2 weeks to Cd (1,250 μg·kg^{-1}) and 17β-estradiol (100 μg·kg^{-1}). Contaminants in a 100 μL volume of NaCl 0,15M were injected in the posterior adductor muscle.

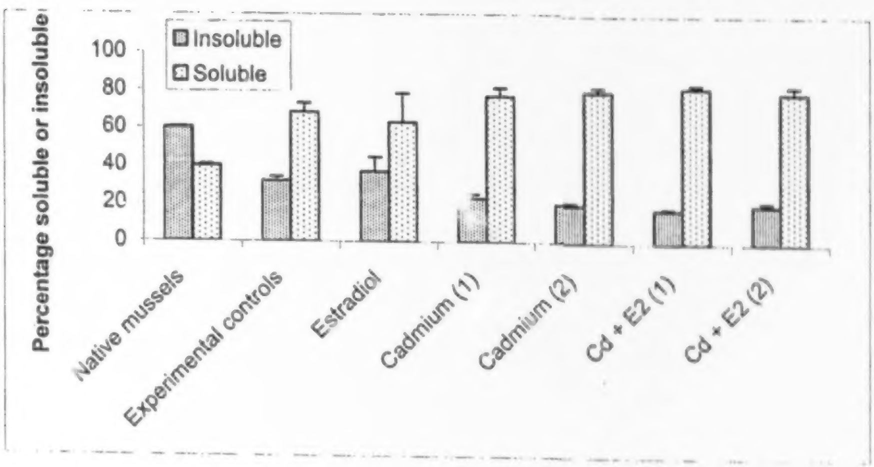

Fig. 3. Partitioning of cadmium concentrations between the soluble and the insoluble fractions in gills of *Mytilus edulis*. Blue mussels were exposed for 2 weeks to Cd (1,250 $\mu g{\cdot}kg^{-1}$) and 17β-estradiol (100 $\mu g{\cdot}kg^{-1}$). Contaminants in a 100 μL volume of NaCl 0,15M were injected in the posterior adductor muscle. Responses were compared between native mussels sampled in an aquaculture plant in Baie de Bourgneuf (France), mussels exposed to NaCl and seawater from the same area (experimental controls), mussels exposed to E2, Cd and Cd + E2. These two groups are represented twice to show differences between duplicates.

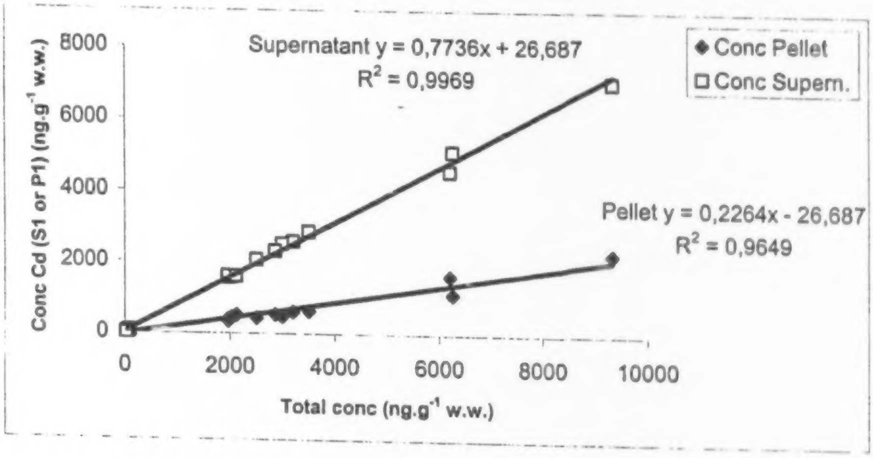

Fig. 4. Partitioning of the soluble and insoluble fractions of Cd and statistical relationship between total Cd concentrations and fractions in gills of *Mytilus edulis*. Blue mussels were exposed for 2 weeks to Cd (1,250 $\mu g{\cdot}kg^{-1}$) and 17β-estradiol (100 $\mu g{\cdot}kg^{-1}$). Contaminants in a 100 μL volume of NaCl 0,15M were injected in the posterior adductor muscle. Responses were compared between native mussels sampled in an aquaculture plant in Baie de Bourgneuf (France), mussels exposed to NaCl and seawater from the same area (experimental controls), mussels exposed to E2, Cd and Cd + E2.

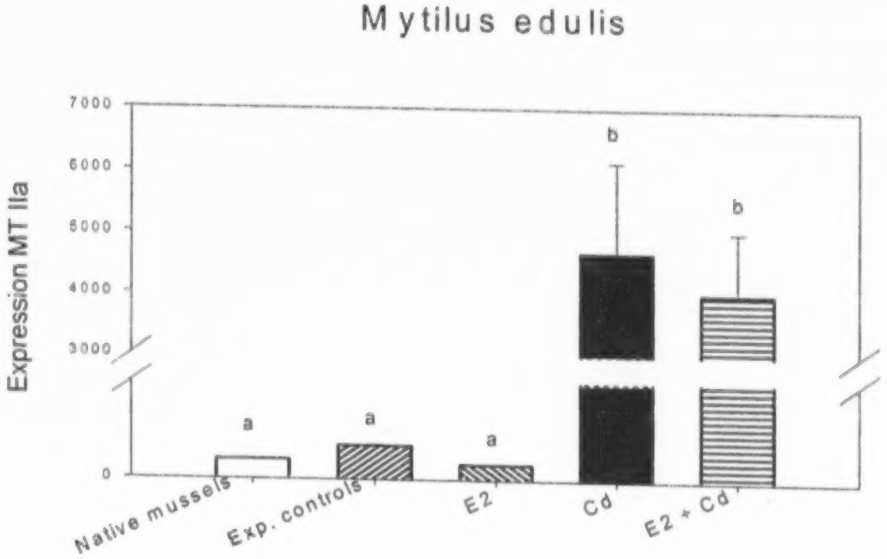

Fig. 5. Comparison of the metallothionein IIa cDNA gene expression in gills of *Mytilus edulis*. Blue mussels were exposed for 2 weeks to Cd (1,250 $\mu g \cdot kg^{-1}$) and 17β-estradiol (100 $\mu g \cdot kg^{-1}$). Contaminants in a 100 μL volume of NaCl 0,15M were injected in the posterior adductor muscle. Responses were compared between native mussels sampled in an aquaculture plant in Baie de Bourgneuf (France), mussels exposed to NaCl and seawater from the same area (experimental controls), mussels exposed to E2, Cd and Cd + E2. Different letters above the bars indicate a significant difference between treatments.

Although it is now well known that MTs are present in *M. edulis* and that it could be induced by Cd exposure, development of genomic tools, responding on the same scale as MT to Cd, will help to understand better the mechanisms of action of this metal and the possible interactions between contaminants. These tools are particularly needed to understand better the importance of contaminants like Cd as endocrine disruptors in bivalves. Recent studies (Gauthier-Clerc et al. 2002, Blaise et al. 1999, Siah et al. 2003) have shown a clear relationship between delays in sexual maturation and decreases in energy reserves and vitellogenin levels in *Mya arenaria*, exposed in situ to contaminants, showing the damage due to endocrine disruptors in marine environments.

Exposure to estradiol as results show in this paper did not modify expression of MT20 IIa cDNA. Estradiol failed also to reduce toxicity of Cd, since increased Cd concentrations were found in the soluble fraction, and did not interact with Cd to increase MT synthesis and gene expression, nor increased toxicity of Cd as it was shown in fish (Olsson et al. 1995). This could possibly be due to the differences between Cd and E2 concentrations used in our study which were far less than those from Olsson et al. (1995) (10 mg E2/kg vs 100 μg/kg and 0,2 mg/kg vs 1,250 μg/kg). The doses chosen for injection in our study correspond to concentrations found in the field in *M. edulis* (Siah et al. 2003) for sites lightly contaminated (RNO 2000). Despite that, these doses were sufficient to induce significantly MTs and gene expression.

Data shown in Fig. 1 clearly show that despite that mussels came from an aquaculture plant, Cd levels were sufficient to synthesize MTs and to induce gene expression. However, Cd was found predominantly in the insoluble fraction, showing that endogenous Cd was stored in lysosomes. Acclimated mussels to experimental basins supplied with filtered seawater used in mussels farms, showed a bioaccumulation of Cd and an inverse pattern of storage between soluble and insoluble fraction when compared to native mussels (Fig. 3). These results show that Cd was kept in the soluble fraction, probably bound to MTs, since increased levels of MTs were measured in Cd exposed animals, correlated to increased accumulation of Cd in the soluble fraction (Fig. 3).

Mussels exposed to E2 and to Cd + E2 showed similar levels of MTs and Cd partitioning, illustrating that E2 did not interfere with or increased MT synthesis or gene expression. Since no data is available in bivalves, our results show that toxicity of estradiol in the presence or absence of Cd in mussels cannot be explained by an enhancement or a decrease of MT synthesis or MT transcription (Polvsen et al. 1990), a result different from those reported in fish (Olsson et al. 1995). Increase of knowledge has been done in this paper about interactions of contaminants on MT synthesis and MTIIa cDNA gene expression as well as showing the similarity of the responses. As it has been shown for MTs (Barsyte et al. 1999, Lemoine et al. 2000) and for key steroidogenic enzymes (Halm et al. 2003), functional genomics constitute sensitive tools that could be used in the near future to understand better threats to aquatic organisms as well as the mechanisms of action of contaminants.

Acknowledgments

The authors would like to thank Professor Khaled Meflah, director of the unit INSERM U 419 for his scientific assistance and Claire Fournier for the analyses of MTs and Cd in tissues of mussels. This work was supported financially by the Individual Discovery Grant Program attributed to Jocelyne Pellerin from NSERC, the Conseil général de Loire-Atlantique and the Université de Nantes.

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Bivalve population status and biomarker responses in *Mya arenaria* clams (Saguenay Fjord, Québec, Canada).

F. Gagné[1], C. Blaise[1] et J. Pellerin[2]. [1]Environnement Canada, Centre Saint-Laurent, Montréal, Qc; et [2]Institut des Sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, Qc.

This study compared population metrics and effects biomarkers in *Mya arenaria* bivalves located at sites subjected to direct sources of contamination with those present at sites having no direct sources of contamination in the Saguenay Fjord and St. Lawrence Estuary (Quebec, Canada). Population parameters included clam density, occurrence of empty shells, age distribution and length-to-age ratio (development index). In addition, two impacted and two reference sites were selected to determine the responses in clams of a suite of biomarkers comprising metallothioneins (MT), lipid peroxidation (LPO), vitellogenin-like proteins(Vtg) and gonado-somatic index (GSI). Results showed that clam populations under direct impact of specific sources of contamination were significantly different, in their population and biomarker profiles, from those at uncontaminated sites. Moreover, animals exhibiting marked responses with MT, LPO, Vtg-like proteins and GSI were collected at impacted sites where clams generally displayed significant changes in development index, clam density and increased mean age values. KEY WORDS: biomarkers, population metrics, bivalves, Saguenay Fjord, St.-Lawrence estuary.

A simple method to evaluate whether a biological community has been influenced by anthropogenic activity. M.F. Bowman[1,2], K.M. Somers[1,2] and R.A. Reid[2]. [1]Trent University, [2]Ontario Ministry of the Environment, Dorset Environmental Science Centre, Dorset, ON.

Introduction

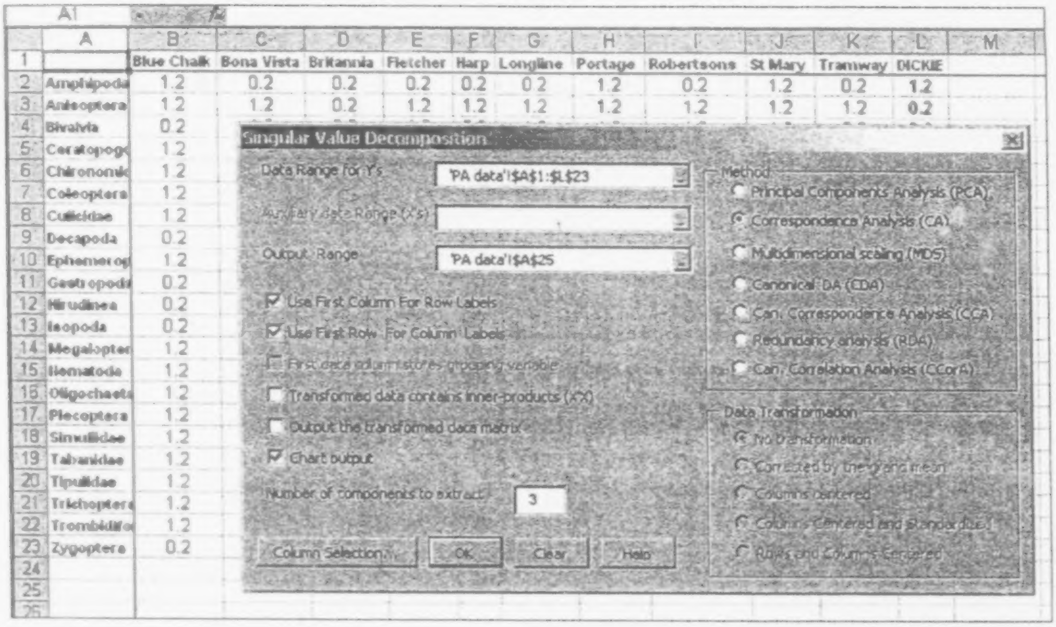
To determine whether the biological community at a test site has been influenced by human activity, the community at the test site can be compared to communities found at minimally impacted reference sites in what is generally called the reference condition approach (e.g., Hughes et al. 1986, Hughes 1995). Currently, there is no consensus on the most effective type of data analysis to use in order to conclude whether a test site has been impacted by anthropogenic activities (Reynoldson and Wright 2000). Numerous indices, each summarizing different aspects of biological condition, are commonly used in bioassessments. However, the techniques used to evaluate test sites with multiple indices or multivariate methods often: (i) involve subjective interpretation, (ii) do not use all biological information available or use redundant information, (iii) are difficult to calculate and explain, and (iv) do not provide probabilities of incorrectly classifying test sites. Herein we demonstrate a test-site analysis (TSA) method that is objective, uses all biological information available, accounts for and identifies redundant information, and therefore, decreases the probability of misclassifying a test site (e.g., see Somers et al. 2003). The TSA method provides a single probability that the test site differs from the reference sites. In addition, a second statistical test can be used to assess whether the test site is impaired to a degree considered ecologically important (Kilgour et al. 1998). Furthermore, our TSA method is applied using Microsoft Excel® and add-ins that are freely available on the internet.

Methods

Sample Dataset: In an assessment of the impacts of acid precipitation on Dorset-area streams in south-central Ontario, MacKay and Kersey (1985) found that macroinvertebrate communities in streams with low pH were less diverse than communities in streams with higher pH. To illustrate the TSA method, we re-sampled one of MacKay and Kersey's acidified streams (Dickie 6) and ten reference streams in May 1999 after high spring flows had receded. All 11 streams are within 35 km of Dorset, are of comparable size (1st and 2nd order) and have similar land-use characteristics (> 90% of the catchment is forested). The pH of all 10 reference streams (Blue Chalk, Bona Vista, Britannia, Fletcher, Harp, Longline, Portage, Robertsons, St. Mary and Tramway) was greater than 6.0. By contrast, the pH of the historically acidified test stream (Dickie) was 4.4.

Benthic macroinvertebrates were collected using a standardized, bioassessment protocol (David et al. 1998). In each stream, three riffles were sampled using a one-minute, kick-and-sweep method (1 m^2 quadrat, 250 μm-mesh D-net). Each sample was sieved in the field and then taken to a laboratory where the debris and associated organisms were randomly subsampled and live sorted until a minimum of 100 animals was obtained. Most organisms were identified to order or coarser taxonomic level, although dipterans were identified to family. Results for the 3 quadrats were combined to produce total counts of approximately 300 organisms for each stream.

Indices: To determine if a test site was impacted, we would normally calculate a number of summary biological indices (10-15) that are appropriate indicators for the suspected stressor(s). Here we selected only 4 metrics to simplify our demonstration of the TSA method. In their original study, MacKay and Kersey (1985) found that in acidic streams, the percentage of plecopterans (i.e., stonefly larvae) was lower, and the percentage of chironomids (i.e., midge larvae) was higher, relative to benthic communities in circum-neutral pH streams. However, Yan et al. (1996) found that multivariate ordination scores were generally better than simple summary metrics as indicators of community



	A	B	C	D	E	F	G	H	I	J	K	L	M
1		Blue Chalk	Bona Vista	Britannia	Fletcher	Harp	Longline	Portage	Robertsons	St Mary	Tramway	DICKIE	
2	Amphipoda	1.2	0.2	0.2	0.2	0.2	0.2	1.2	0.2	1.2	0.2	1.2	
3	Anisoptera	1.2	1.2	0.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.2	
4	Bivalvia	0.2											
5	Ceratopog	1.2											
6	Chironomid	1.2											
7	Coleoptera	1.2											
8	Culicidae	1.2											
9	Decapoda	0.2											
10	Ephemerop	1.2											
11	Gastropod	0.2											
12	Hirudinea	0.2											
13	Isopoda	0.2											
14	Megalopter	1.2											
15	Nematoda	1.2											
16	Oligochaet	1.2											
17	Plecoptera	1.2											
18	Simuliidae	1.2											
19	Tabanidae	1.2											
20	Tipulidae	1.2											
21	Trichopter	1.2											
22	Trombidifo	1.2											
23	Zygoptera	0.2											
24													
25													

Singular Value Decomposition

Data Range for Y's: 'PA data'!$A$1:$L$23

Auxiliary data Range (X's):

Output Range: 'PA data'!$A$25

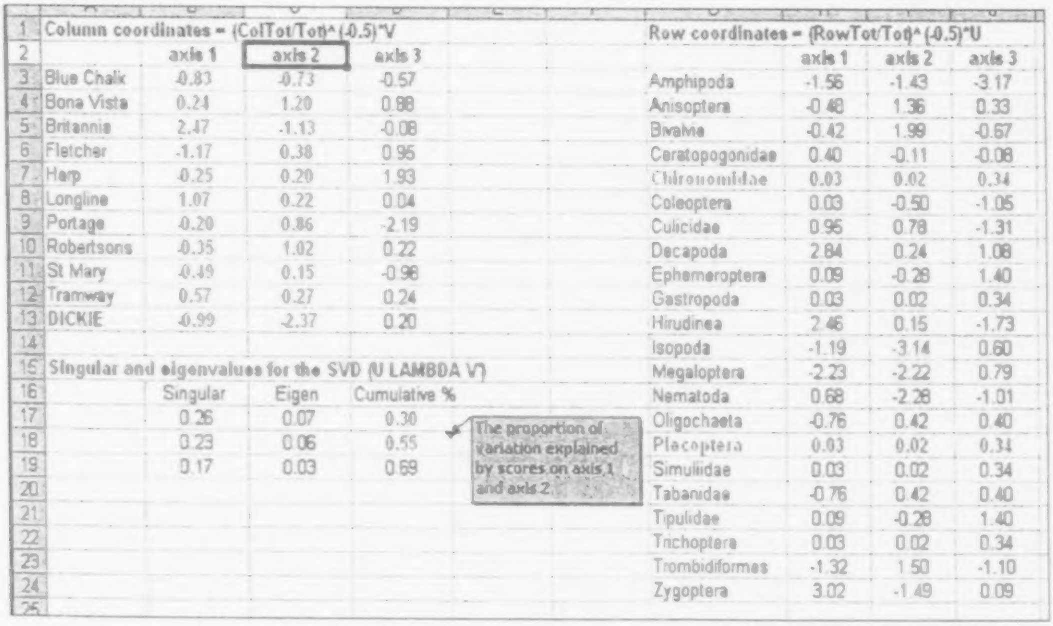
☑ Use First Column For Row Labels
☑ Use First Row For Column Labels
☐ First data column stores grouping variable
☐ Transformed data contains inner-products (X'X)
☐ Output the transformed data matrix
☑ Chart output

Number of components to extract: 3

Method
○ Principal Components Analysis (PCA)
● Correspondence Analysis (CA)
○ Multidimensional scaling (MDS)
○ Canonical DA (CDA)
○ Can. Correspondence Analysis (CCA)
○ Redundancy analysis (RDA)
○ Can. Correlation Analysis (CCorA)

Data Transformation
● No transformation
○ Corrected by the grand mean
○ Columns centered
○ Columns Centered and Standardized
○ Rows and Columns Centered

Fig.1. Input information required to perform a correspondence analysis in Biplot.



Column coordinates = (ColTot/Tot)^(-0.5)*V

	axis 1	axis 2	axis 3
Blue Chalk	-0.83	-0.73	-0.57
Bona Vista	0.24	1.20	0.88
Britannia	2.47	-1.13	-0.08
Fletcher	-1.17	0.38	0.95
Harp	-0.25	0.20	1.93
Longline	1.07	0.22	0.04
Portage	-0.20	0.86	-2.19
Robertsons	-0.35	1.02	0.22
St Mary	-0.49	0.15	-0.98
Tramway	0.57	0.27	0.24
DICKIE	-0.99	-2.37	0.20

Singular and eigenvalues for the SVD (U LAMBDA V')

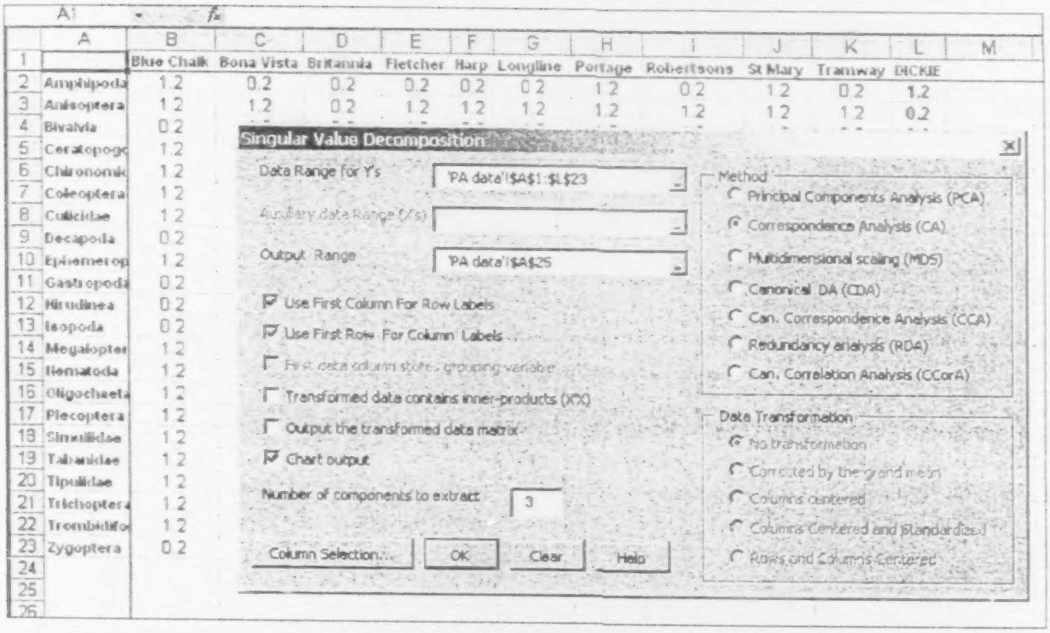
Singular	Eigen	Cumulative %
0.26	0.07	0.30
0.23	0.06	0.55
0.17	0.03	0.69

The proportion of variation explained by scores on axis 1 and axis 2

Row coordinates = (RowTot/Tot)^(-0.5)*U

	axis 1	axis 2	axis 3
Amphipoda	-1.56	-1.43	-3.17
Anisoptera	-0.48	1.36	0.33
Bivalvia	-0.42	1.99	-0.67
Ceratopogonidae	0.40	-0.11	-0.08
Chironomidae	0.03	0.02	0.34
Coleoptera	0.03	-0.50	-1.05
Culicidae	0.95	0.78	-1.31
Decapoda	2.84	0.24	1.08
Ephemeroptera	0.09	-0.28	1.40
Gastropoda	0.03	0.02	0.34
Hirudinea	2.46	0.15	-1.73
Isopoda	-1.19	-3.14	0.60
Megaloptera	-2.23	-2.22	0.79
Nematoda	0.68	-2.28	-1.01
Oligochaeta	-0.76	0.42	0.40
Plecoptera	0.03	0.02	0.34
Simuliidae	0.03	0.02	0.34
Tabanidae	-0.76	0.42	0.40
Tipulidae	0.09	-0.28	1.40
Trichoptera	0.03	0.02	0.34
Trombidiformes	-1.32	1.50	-1.10
Zygoptera	3.02	-1.49	0.09

Fig. 2. Output from a correspondence analysis performed in Biplot.



	Blue Chalk	Bona Vista	Britannia	Fletcher	Harp	Longline	Portage	Robertsons	St Mary	Tramway	DICKIE
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Anisoptera	1.2	1.2	0.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.2
Bivalvia	0.2										
Ceratopogc	1.2										
Chironomic	1.2										
Coleoptera	1.2										
Culicidae	1.2										
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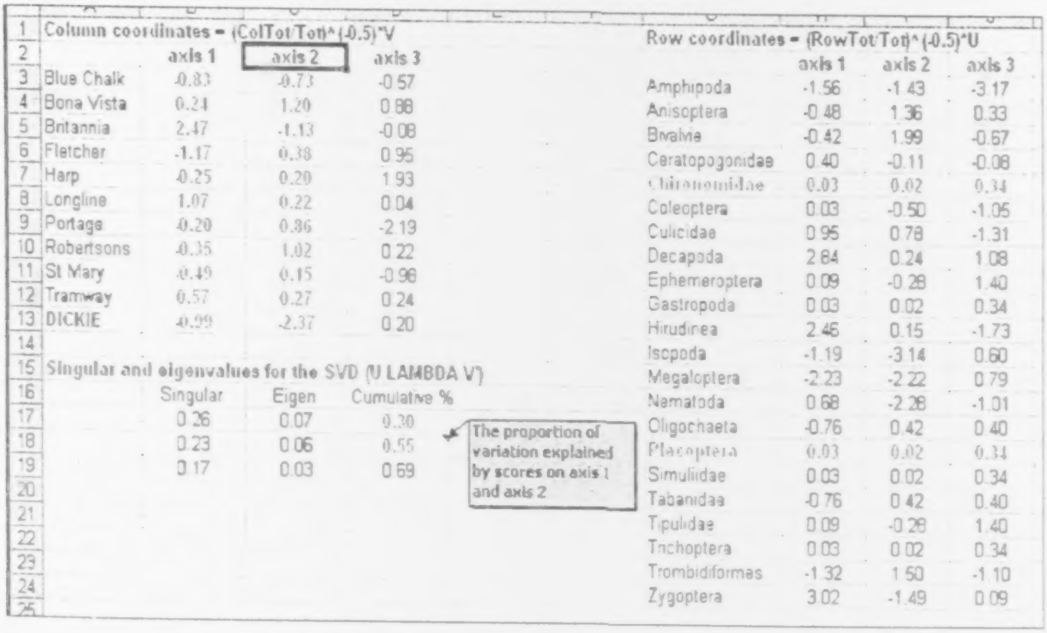

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Trombidiformes	-1.32	1.50	-1.10
Zygoptera	3.02	-1.49	0.09

Fig. 2. Output from a correspondence analysis performed in Biplot.

Table 1. Sequence of calculations used in each step of TEST SITE ANALYSIS (TSA)

STEP 1. Calculate Indices that will Characterize Biological Community Structure	
[a]Appropriate metrics	e.g., %Plecoptera, %Chironomidae (Barbour et al. 1999)
[b]CA axes	e.g., CA1, CA2 (Fig.1&2)
STEP 2. Calculate the Generalized Distance (D) Between Reference Site Mean and Test Site	
[a]standardize data	$(\text{value}_{test} - \text{mean}_{reference})$ / standard deviation$_{reference}$ (Fig.5)
[a]covariance matrix	Tools/Data analyses/covariance on standardized data (Fig.6)
[a]matrix inverse	**MINVERSE** (full covariance matrix) (Fig.6)
[a]matrix products	**MMULT** (standardized test data , inverse matrix) (Fig.7)
	MMULT (resultant product, transposed test data)
STEP 3. Assess the Statistical Significance of D (using central and / or non-central tests)	
Numerator df	number of indices (p)
Denominator df	number of reference sites (n_{ref}) – number of indices
[a]F	$((n_{ref} - p) * n_{ref} * D^2) / (p * (n_{ref} - 1))$
[a]Central P	**FDIST** (F, p, n_{ref} - p)
[a]χ^2	**CHIINV** (0.05, p)
[a]λ	$\chi^2 * n_{ref}$
[c]Non-central P	1 - (**NCF** (F, p, n_{ref} - p, λ, 1e-8, 400))
STEP 4. Determine the Contribution of Each Index to the TSA (in uni- and multivariate analyses)	
Univariate	
[a]Standardized difference (δ)	$(\text{value}_{test} - \text{mean}_{reference})$ / standard deviation$_{reference}$
[a]t	δ * **SQRT** (n_{ref})
[a]P	**TDIST** (**ABS** (t), n_{ref} - 1, 2)
Multivariate	
[a]T^2	$n_{ref} * D^2$
[a]Partial T^2 (pT^2)	redo calculations for D, omitting one index at a time
	SQRT $((n_{ref} - p) * (T^2 - pT^2) / (n_{ref} + pT^2))$
[a]Partial F	$pT^2 * pT^2$
[a]P	FDIST(F, 1, n_{ref} - p)

---

[a] standard excel worksheet functions (in bold) required (Microsoft Corporation 2003)

[b] biplot add-in required (Lipkovich and Smith 2001)

[c] pie-face add-in required (Lenth 2003)

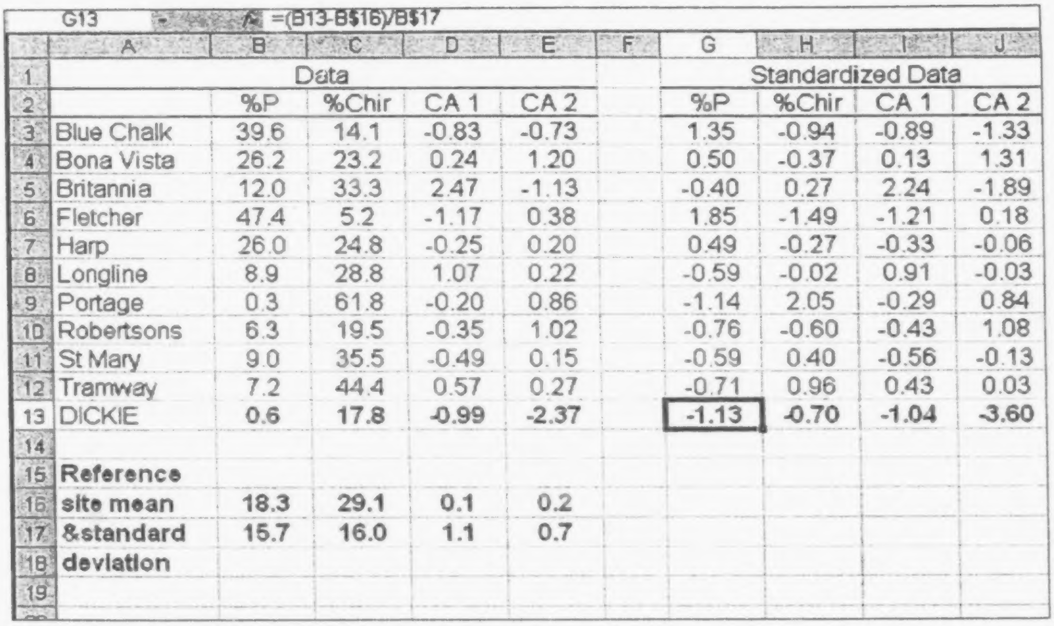
change. Therefore, we selected the following indices: (i) the total number of Plecoptera and the total number of Chironomidae found in the 3 quadrats divided by the total number of organisms counted for each stream, and (ii) the first and second axis scores of a correspondence analysis (CA) ordination of the streams-by-taxa, presence-absence data.

Multivariate ordinations are often used to summarize large matrices of sites-by-taxa data into a smaller set of axis scores that represent the dominant trends of variation among sites. Of the various types of ordinations, correspondence analysis (CA) is appropriate for abundance and presence-absence (P/A) data (Legendre and Legendre 1998). When CA is used to summarize P/A data, the resultant scores generally reflect patterns in community richness associated with species occurrence and co-occurrence. Because CAs can be strongly influenced by rare taxa, the rare taxa are generally removed or down-weighted prior to analysis (ter Braak and Prentice 1988). Here, we used the full P/A data matrix with rare taxa down-weighted by adding 0.2 to all values in the matrix (e.g., see Keller et al. 2002). The CA ordination was calculated in a simple spreadsheet (Figs. 1 and 2) using the Biplot add-in for Excel® (Lipkovich and Smith 2001). The resultant CA bi-plot shows the relative positions of the reference and test streams as well as the taxa that are important in defining the first and second axes (Figs. 3 and 4).

Generalized Distance (**D**): In order to estimate the biological similarity among test and reference streams using all summary indices simultaneously, we calculated the generalized or Mahalanobis distance (e.g., Legendre and Legendre 1998). The generalized distance (**D**) is a standardized Euclidean distance that accounts for correlations or redundancies among indices. By using **D**, the estimated biological distance among streams is not biased by our choice of metrics if these indices measure redundant aspects of the benthic community. In this demonstration, **D** is calculated using information associated with 4 indices (i.e., the number of variables, p=4).

To calculate generalized distance, all data associated with each summary biological index were centred by subtracting the average value for the 10 reference streams and then standardized by dividing by the standard deviation associated with the 10 reference streams (Table 1, Fig. 5). The standardization step was included because many biological indices are measured in different units (or on different scales) and this step weights the indices equally in the analysis. The standardized data for the reference streams were used to calculate a variance-covariance matrix among the 4 metrics (Table 1, Fig. 6 - matrix 1). The resultant 4 x 4 variance-covariance matrix ($\mathbf{S}_{ref}$ - Fig. 6 - matrix 2) was used to calculate an inverse matrix ($\mathbf{S}^{-1}_{ref}$ - Fig. 6 - matrix 3) and the vector of standardized values for the test stream ($[test - \mathbf{X}_{ref}]/\mathbf{SD}_{ref}$ - Fig. 6 – matrix 4) was multiplied by the inverse matrix. Subsequently, $\mathbf{D}^2$ was calculated by multiplying the resultant product (Fig. 6 – matrix 5) by the transposed vector associated with the test stream (($[test - \mathbf{X}_{ref}]/\mathbf{SD}_{ref}$)**N** - Fig. 6 – transposed matrix 4). Thus, **D** was calculated using the following equation:

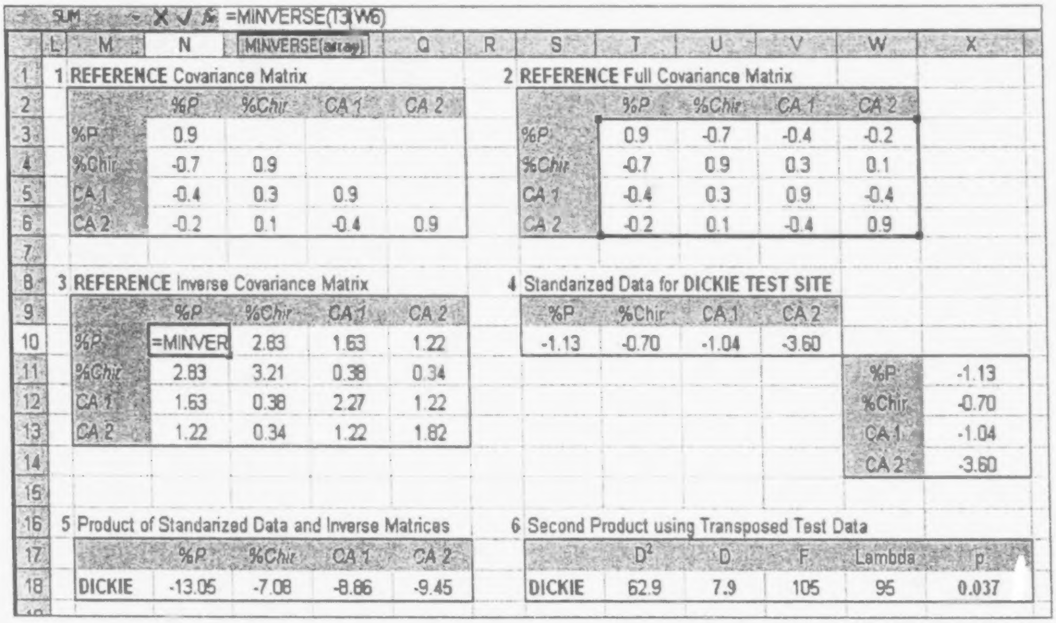
$$D = \sqrt{\left(\frac{test - \overline{X}_{ref}}{SD_{ref}}\right) * S^{-1}_{ref} * \left(\frac{test - \overline{X}_{ref}}{SD_{ref}}\right)^{\prime}}$$



G13 =(B13-B$16)/B$17

	A	B	C	D	E	F	G	H	I	J
1		Data					Standardized Data			
2		%P	%Chir	CA 1	CA 2		%P	%Chir	CA 1	CA 2
3	Blue Chalk	39.6	14.1	-0.83	-0.73		1.35	-0.94	-0.89	-1.33
4	Bona Vista	26.2	23.2	0.24	1.20		0.50	-0.37	0.13	1.31
5	Britannia	12.0	33.3	2.47	-1.13		-0.40	0.27	2.24	-1.89
6	Fletcher	47.4	5.2	-1.17	0.38		1.85	-1.49	-1.21	0.18
7	Harp	26.0	24.8	-0.25	0.20		0.49	-0.27	-0.33	-0.06
8	Longline	8.9	28.8	1.07	0.22		-0.59	-0.02	0.91	-0.03
9	Portage	0.3	61.8	-0.20	0.86		-1.14	2.05	-0.29	0.84
10	Robertsons	6.3	19.5	-0.35	1.02		-0.76	-0.60	-0.43	1.08
11	St Mary	9.0	35.5	-0.49	0.15		-0.59	0.40	-0.56	-0.13
12	Tramway	7.2	44.4	0.57	0.27		-0.71	0.96	0.43	0.03
13	DICKIE	**0.6**	**17.8**	**-0.99**	**-2.37**		**-1.13**	**-0.70**	**-1.04**	**-3.60**
14										
15	**Reference**									
16	**site mean**	**18.3**	**29.1**	**0.1**	**0.2**					
17	**&standard**	**15.7**	**16.0**	**1.1**	**0.7**					
18	**deviation**									
19										

Fig. 5. Raw index values, and index values standardized by subtracting the reference site mean and dividing by the reference site standard deviation (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



SUM =MINVERSE(T3:W6)

1 **REFERENCE** Covariance Matrix

	%P	%Chir	CA 1	CA 2
%P	0.9			
%Chir	-0.7	0.9		
CA 1	-0.4	0.3	0.9	
CA 2	-0.2	0.1	-0.4	0.9

2 **REFERENCE** Full Covariance Matrix

	%P	%Chir	CA 1	CA 2
%P	0.9	-0.7	-0.4	-0.2
%Chir	-0.7	0.9	0.3	0.1
CA 1	-0.4	0.3	0.9	-0.4
CA 2	-0.2	0.1	-0.4	0.9

3 **REFERENCE** Inverse Covariance Matrix

	%P	%Chir	CA 1	CA 2
%P	=MINVER	2.83	1.63	1.22
%Chir	2.83	3.21	0.38	0.34
CA 1	1.63	0.38	2.27	1.22
CA 2	1.22	0.34	1.22	1.82

4 Standarized Data for **DICKIE TEST SITE**

%P	%Chir	CA 1	CA 2
-1.13	-0.70	-1.04	-3.60

%P	-1.13
%Chir	-0.70
CA 1	-1.04
CA 2	-3.60

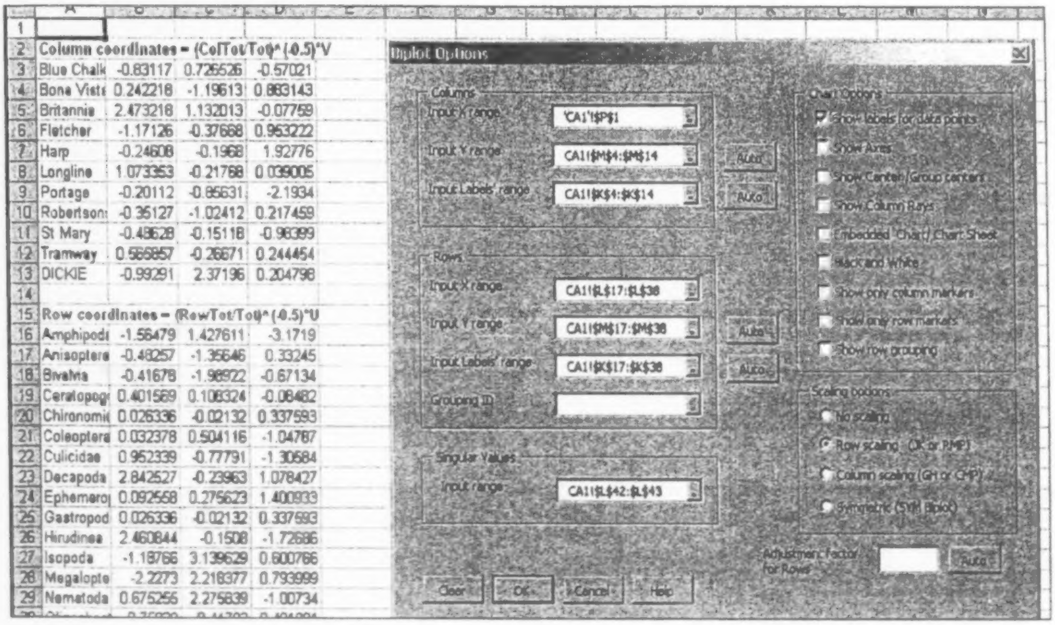
5 Product of Standarized Data and Inverse Matrices

	%P	%Chir	CA 1	CA 2
DICKIE	-13.05	-7.08	-8.86	-9.45

6 Second Product using Transposed Test Data

	D^2	D	F	Lambda	p
DICKIE	62.9	7.9	105	95	0.037

Fig. 6. Six matrices used to calculate the generalized distance (D) between a test site and the reference site mean (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



	A	B	C	D
1				
2	**Column coordinates = (ColTot/Tot)^(-0.5)*V**			
3	Blue Chalk	-0.83117	0.726526	-0.57021
4	Bona Vista	0.242218	-1.19613	0.883143
5	Britannia	2.473218	1.132013	-0.07759
6	Fletcher	-1.17126	-0.37668	0.963222
7	Harp	-0.24608	-0.1968	1.92776
8	Longline	1.073353	-0.21768	0.039005
9	Portage	-0.20112	-0.85631	-2.1934
10	Robertsons	-0.35127	-1.02412	0.217459
11	St Mary	-0.48628	-0.15118	-0.98399
12	Tramway	0.565857	-0.26671	0.244454
13	DICKIE	-0.99291	2.37196	0.204798
14				
15	**Row coordinates = (RowTot/Tot)^(-0.5)*U**			
16	Amphipoda	-1.56479	1.427611	-3.1719
17	Anisoptera	-0.48257	-1.35646	0.33245
18	Bivalvia	-0.41678	-1.98922	-0.67134
19	Ceratopogonidae	0.401569	0.108324	-0.08482
20	Chironomidae	0.026336	-0.02132	0.337593
21	Coleoptera	0.032378	0.504116	-1.04787
22	Culicidae	0.952339	-0.77791	-1.30584
23	Decapoda	2.842527	-0.23963	1.078427
24	Ephemeroptera	0.092568	0.275623	1.400933
25	Gastropoda	0.026336	-0.02132	0.337593
26	Hirudinea	2.460844	-0.1508	-1.72686
27	Isopoda	-1.18766	3.139629	0.600766
28	Megaloptera	-2.2273	2.218377	0.793999
29	Nematoda	0.675256	2.275639	-1.00734

Biplot Options

Columns
Input X range: 'CA1'!$P$1
Input Y range: CA1!$M$4:$M$14
Input Labels' range: CA1!$K$4:$K$14

Rows
Input X range: CA1!$L$17:$L$38
Input Y range: CA1!$M$17:$M$38
Input Labels' range: CA1!$K$17:$K$38
Grouping ID:

Singular Values
Input range: CA1!$L$42:$L$43

Chart Options
☑ Show labels for data points
☐ Show Axes
☐ Show Center/Group centers
☐ Show Column Rays
☐ Embedded Chart/Chart Sheet
☐ Black and White
☐ Show only column markers
☐ Show only row markers
☐ Show row grouping

Scaling options
○ No scaling
● Row scaling (JK or RMP)
○ Column scaling (GH or CMP)
○ Symmetric (SYM Biplot)

Adjustment Factor for Rows:

Fig. 3. Graphing window and input information that automatically appears in Biplot following a correspondence analysis.

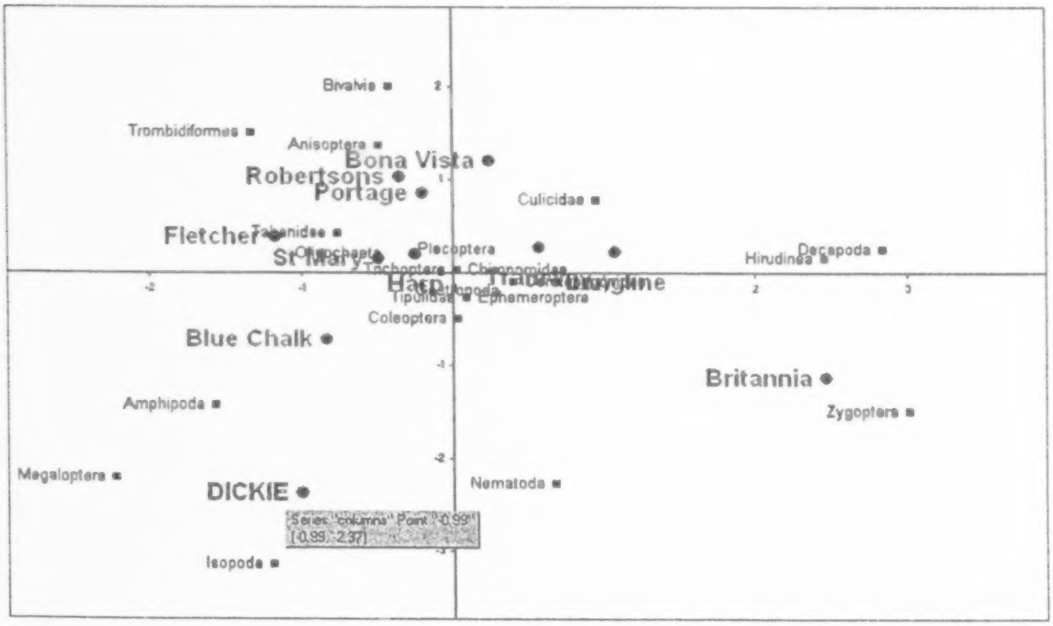
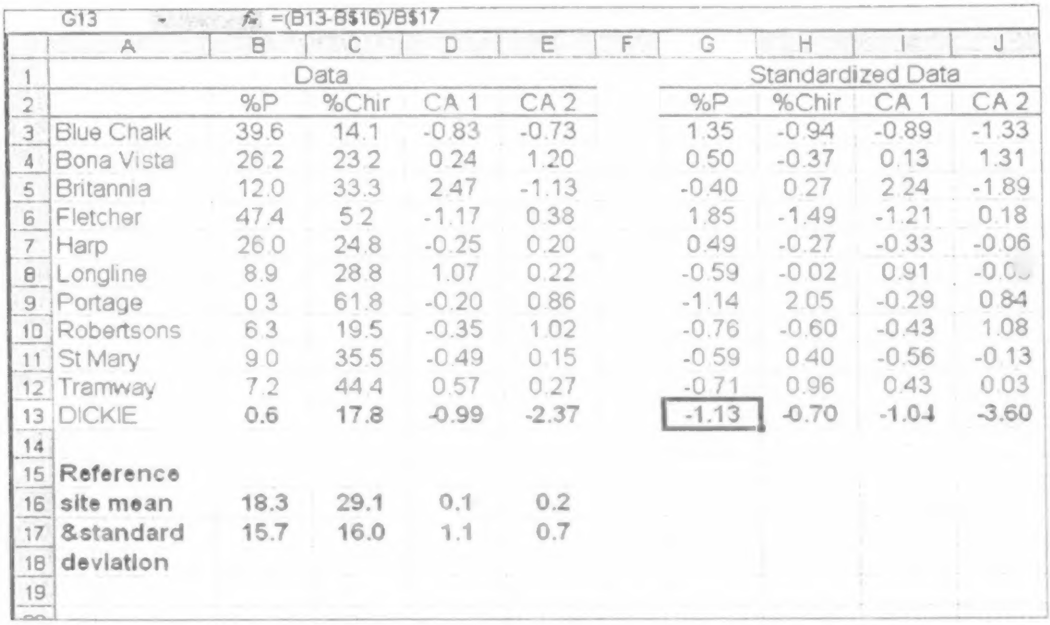


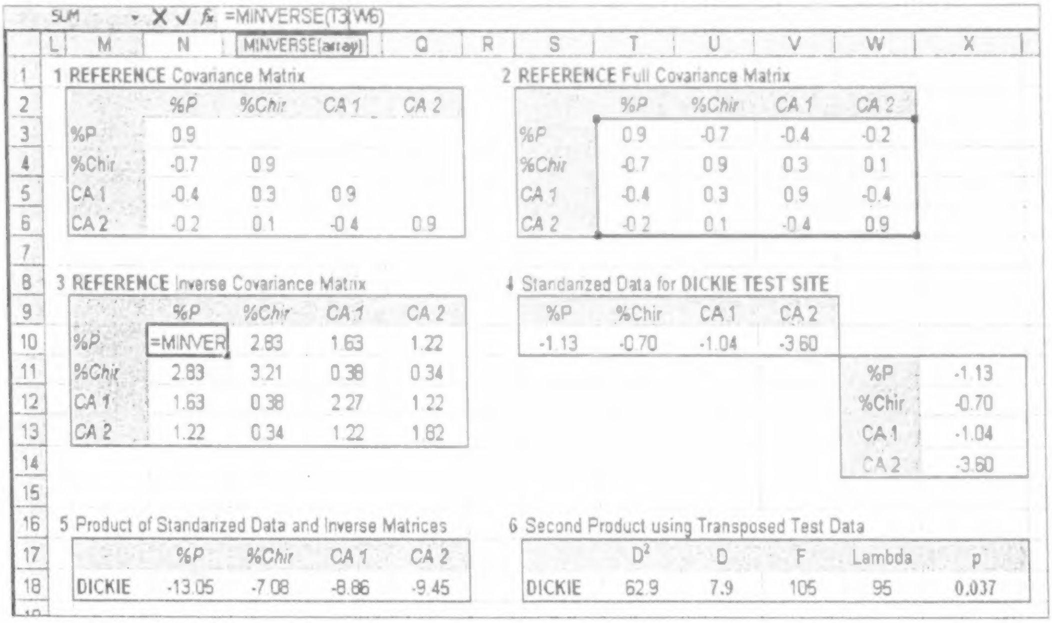
Fig. 4. Graph of correspondence analysis (CA) axes one versus axes two, showing the position of the Dickie test site relative to reference sites, and the taxa important in defining the CA axes.



G13 =(B13-B$16)/B$17

	A	B	C	D	E	F	G	H	I	J
1		Data					Standardized Data			
2		%P	%Chir	CA 1	CA 2		%P	%Chir	CA 1	CA 2
3	Blue Chalk	39.6	14.1	-0.83	-0.73		1.35	-0.94	-0.89	-1.33
4	Bona Vista	26.2	23.2	0.24	1.20		0.50	-0.37	0.13	1.31
5	Britannia	12.0	33.3	2.47	-1.13		-0.40	0.27	2.24	-1.89
6	Fletcher	47.4	5.2	-1.17	0.38		1.85	-1.49	-1.21	0.18
7	Harp	26.0	24.8	-0.25	0.20		0.49	-0.27	-0.33	-0.06
8	Longline	8.9	28.8	1.07	0.22		-0.59	-0.02	0.91	-0.0[illegible]
9	Portage	0.3	61.8	-0.20	0.86		-1.14	2.05	-0.29	0.84
10	Robertsons	6.3	19.5	-0.35	1.02		-0.76	-0.60	-0.43	1.08
11	St Mary	9.0	35.5	-0.49	0.15		-0.59	0.40	-0.56	-0.13
12	Tramway	7.2	44.4	0.57	0.27		-0.71	0.96	0.43	0.03
13	DICKIE	**0.6**	**17.8**	**-0.99**	**-2.37**		-1.13	**-0.70**	**-1.04**	**-3.60**
14										
15	**Reference**									
16	**site mean**	**18.3**	**29.1**	**0.1**	**0.2**					
17	**&standard**	**15.7**	**16.0**	**1.1**	**0.7**					
18	**deviation**									
19										

Fig. 5. Raw index values, and index values standardized by subtracting the reference site mean and dividing by the reference site standard deviation (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



SUM =MINVERSE(T3:W6)

L M N MINVERSE(array) Q R S T U V W X

1 **REFERENCE** Covariance Matrix

	%P	%Chir	CA 1	CA 2
%P	0.9			
%Chir	-0.7	0.9		
CA 1	-0.4	0.3	0.9	
CA 2	-0.2	0.1	-0.4	0.9

2 **REFERENCE** Full Covariance Matrix

	%P	%Chir	CA 1	CA 2
%P	0.9	-0.7	-0.4	-0.2
%Chir	-0.7	0.9	0.3	0.1
CA 1	-0.4	0.3	0.9	-0.4
CA 2	-0.2	0.1	-0.4	0.9

3 **REFERENCE** Inverse Covariance Matrix

	%P	%Chir	CA 1	CA 2
%P	=MINVER	2.83	1.63	1.22
%Chir	2.83	3.21	0.38	0.34
CA 1	1.63	0.38	2.27	1.22
CA 2	1.22	0.34	1.22	1.82

4 Standarized Data for **DICKIE TEST SITE**

%P	%Chir	CA 1	CA 2
-1.13	-0.70	-1.04	-3.60

%P	-1.13
%Chir	-0.70
CA 1	-1.04
CA 2	-3.60

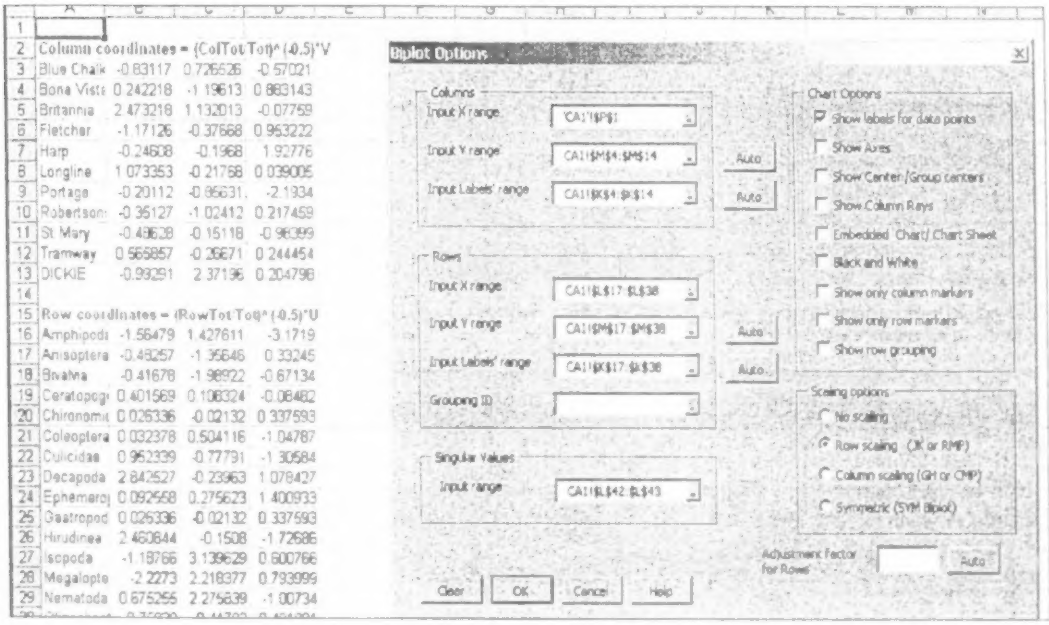
5 Product of Standarized Data and Inverse Matrices

	%P	%Chir	CA 1	CA 2
DICKIE	-13.05	-7.08	-8.86	-9.45

6 Second Product using Transposed Test Data

	D^2	D	F	Lambda	p
DICKIE	62.9	7.9	105	95	0.037

Fig. 6. Six matrices used to calculate the generalized distance (D) between a test site and the reference site mean (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



	A	B	C	D
1				
2	Column coordinates = (ColTot/Tot)^(-0.5)*V			
3	Blue Chalk	-0.83117	0.726526	-0.57021
4	Bona Vista	0.242218	-1.19613	0.883143
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9	Portage	-0.20112	-0.86631	-2.1934
10	Robertsons	-0.35127	-1.02412	0.217459
11	St Mary	-0.48628	-0.15118	-0.98399
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13	DICKIE	-0.93291	2.37136	0.204796
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16	Amphipoda	-1.56479	1.427611	-3.1719
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22	Culicidae	0.952339	-0.77731	-1.30584
23	Decapoda	2.842527	-0.23963	1.078427
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25	Gastropod	0.026336	-0.02132	0.337593
26	Hirudinea	2.460844	-0.1508	-1.72686
27	Isopoda	-1.18766	3.139629	0.600766
28	Megalopte	-2.2273	2.218377	0.793999
29	Nematoda	0.675255	2.275639	-1.00734

Columns
Input X range: 'CA1'!$P$1
Input Y range: CA1!$M$4:$M$14
Input Labels' range: CA1!$K$4:$K$14

Rows
Input X range: CA1!$L$17:$L$38
Input Y range: CA1!$M$17:$M$38
Input Labels' range: CA1!$K$17:$K$38
Grouping ID:

Singular Values
Input range: CA1!$L$42:$L$43

Chart Options
☑ Show labels for data points
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☐ Show Center /Group centers
☐ Show Column Rays
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☐ Show only row markers
☐ Show row grouping

Scaling options
○ No scaling
● Row scaling (JK or RMP)
○ Column scaling (GH or CMP)
○ Symmetric (SYM Biplot)

Adjustment Factor for Rows:

Fig. 3. Graphing window and input information that automatically appears in Biplot following a correspondence analysis.

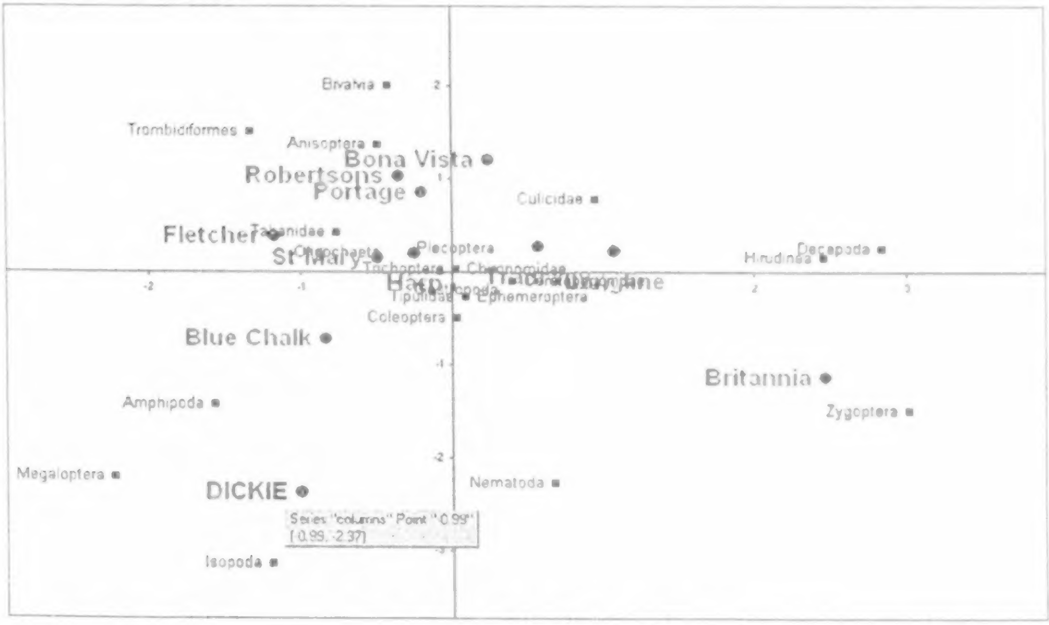


Fig. 4. Graph of correspondence analysis (CA) axes one versus axes two, showing the position of the Dickie test site relative to reference sites, and the taxa important in defining the CA axes.

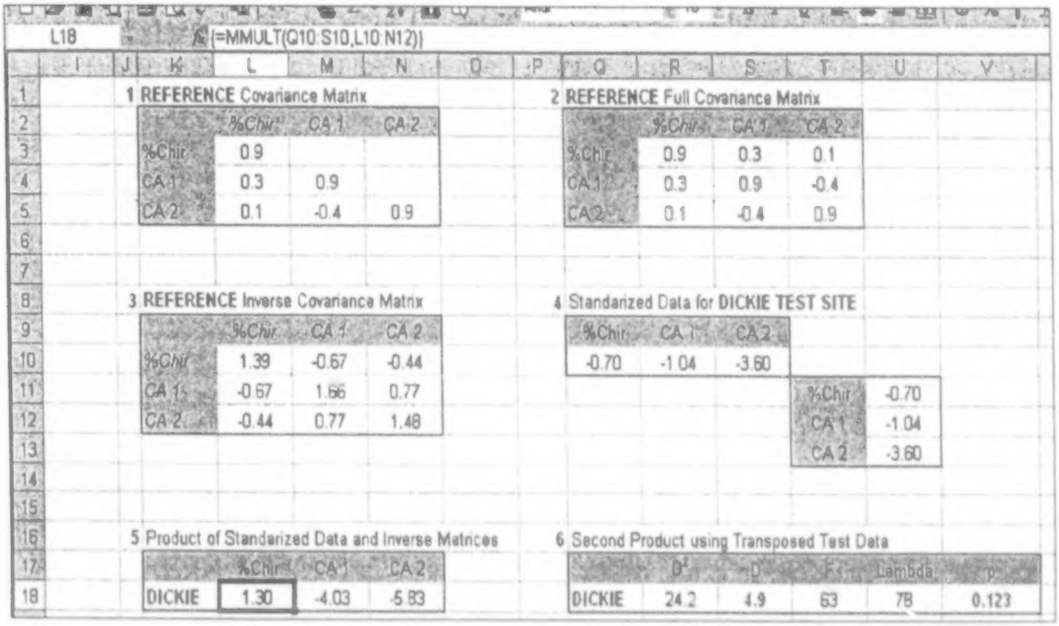
To determine whether the biological condition of the test stream was significantly different from the reference-stream mean, we could evaluate whether the distance **D** was significantly different from zero. If this test is based on $\mathbf{D}^2$, then $\mathbf{D}^2$ multiplied by the number of reference streams (n_{ref}) approximates the standard multivariate $\mathbf{T}^2$ test (Legendre and Legendre 1998). The significance of $\mathbf{D}^2$ is assessed using an **F** value calculated as:

$$F = \frac{\left(\left(n_{ref} - p\right) * n_{ref} * D^2\right)}{\left(p * \left(n_{ref} - 1\right)\right)} \quad \text{with } p \text{ and } (n_{ref} - p) \text{ df.}$$

In this **F** test, we are evaluating whether the difference (or biological distance) between the test stream and the reference-stream mean is significantly different from zero. Because statistical significance is affected by power (i.e., significance is a function of the number of reference streams and number of metrics, as well as the effect size), Kilgour et al. (1998) recommended that we focus on whether there is an ecologically meaningful rather than a statistically significant difference between the test and reference streams. As a result, Kilgour et al. proposed that the observed difference between the test stream and the reference-stream mean should be significantly greater than the normal range of variation among the reference streams. Kilgour et al. defined the normal range as the confidence region enclosing 95% of the reference streams, and hence, the appropriate test assesses whether the test stream is significantly outside of the normal range. That is, we test whether the difference between the test stream and the reference-stream mean is greater than the normal range instead of the traditional test that the observed difference is greater than zero. This type of statistical test is a non-central test, whereas the traditional test evaluating a difference of zero is a central test.

To statistically evaluate a non-central test, the critical difference (or critical effect size) must be defined *a priori*. Following Kilgour et al. (1998), we based our critical effect size on the normal range of variation among reference streams. In non-central tests, this effect size is typically expressed as a function of the non-centrality parameter (ë). Because we are using the generalized distance, the non-centrality parameter associated with the distance enclosing 95% of the reference-stream observations is defined by the 95th percentage point of the chi-square distribution with *p* df, where *p* is the number of metrics used to calculate the generalized distance (i.e., $\lambda = \div\chi^2_{(0.05,p)} * n_{\text{ref}}$; Kilgour et al. 1998). Having determined the non-centrality parameter, the probability that a test stream lies significantly outside of the normal range is readily calculated using the *πface* add-in for Excel® (Lenth 2003) and the observed F value defined above. As a result, the probability associated with the traditional **F** test indicates whether the test stream is significantly different from the reference-stream mean (i.e., $\mathbf{D} \neq 0$), and the non-central test probability indicates whether the test stream lies significantly outside of the normal range of variation among the reference streams (i.e., **D** > normal range).

To determine the relative importance of each biological index in separating the test stream from the reference streams, calculations for **D** and $\mathbf{T}^2$ can be repeated p times leaving out a different metric each time. The differences between the original analysis and the analyses using one fewer index are used to calculate *partial* $\mathbf{T}^2$ values for each index that is omitted (e.g., Rencher and Scott 1990, Table 1). The *partial* $\mathbf{T}^2$ values indicate the amount of unique information that a given index adds to the analysis given the variation already explained by the other metrics. The index with the highest *partial* $\mathbf{T}^2$ contributed the most unique information to the multivariate assessment (Table 1, Figs.7 and 8), whereas metrics with small *partial* $\mathbf{T}^2$ values add very little to the analysis.



L18 {=MMULT(Q10:S10,L10:N12)}

1 REFERENCE Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	0.9		
CA 1	0.3	0.9	
CA 2	0.1	-0.4	0.9

2 REFERENCE Full Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	0.9	0.3	0.1
CA 1	0.3	0.9	-0.4
CA2	0.1	-0.4	0.9

3 REFERENCE Inverse Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	1.39	-0.67	-0.44
CA 1	-0.67	1.66	0.77
CA 2	-0.44	0.77	1.48

4 Standarized Data for DICKIE TEST SITE

%Chir	CA 1	CA 2
-0.70	-1.04	-3.60

%Chir	-0.70
CA 1	-1.04
CA 2	-3.60

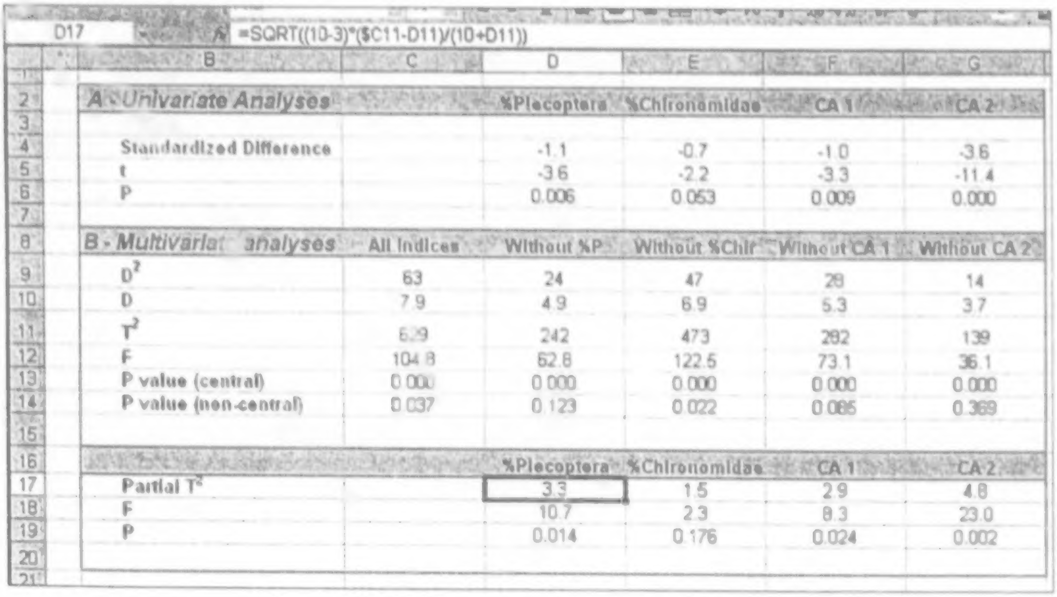
5 Product of Standarized Data and Inverse Matrices

	%Chir	CA 1	CA 2
DICKIE	1.30	-4.03	-5.83

6 Second Product using Transposed Test Data

	D^2	D	F	Lambda	p
DICKIE	24.2	4.9	63	78	0.123

Fig. 7. Initial six steps used to calculate partial T^2 for % Plecoptera metric (Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



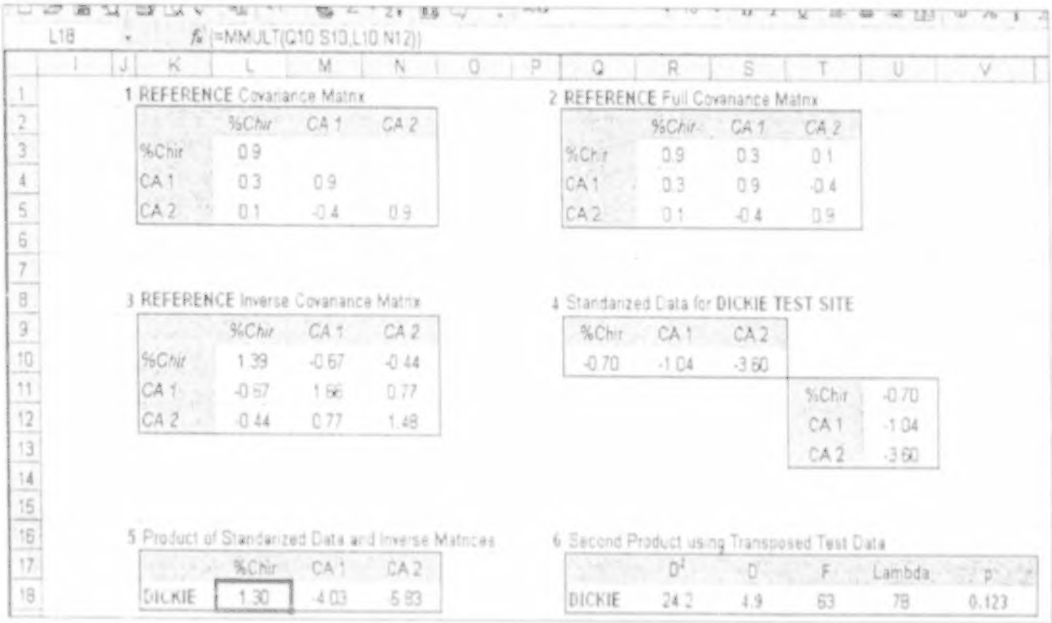
D17 =SQRT((10-3)*($C11-D11)/(10+D11))

A - Univariate Analyses		%Plecoptera	%Chironomidae	CA 1	CA 2
Standardized Difference		-1.1	-0.7	-1.0	-3.6
t		-3.6	-2.2	-3.3	-11.4
P		0.006	0.053	0.009	0.000

B - Multivariat analyses	All Indices	Without %P	Without %Chir	Without CA 1	Without CA 2
D^2	63	24	47	28	14
D	7.9	4.9	6.9	5.3	3.7
T^2	629	242	473	282	139
F	104.8	62.8	122.5	73.1	36.1
P value (central)	0.000	0.000	0.000	0.000	0.000
P value (non-central)	0.037	0.123	0.022	0.086	0.369

		%Plecoptera	%Chironomidae	CA 1	CA 2
Partial T^2		3.3	1.5	2.9	4.8
F		10.7	2.3	8.3	23.0
P		0.014	0.176	0.024	0.002

Fig. 8. Summary of TSA results (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



L18 {=MMULT(Q10:S10,L10:N12)}

1 **REFERENCE** Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	0.9		
CA 1	0.3	0.9	
CA 2	0.1	-0.4	0.9

2 **REFERENCE** Full Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	0.9	0.3	0.1
CA 1	0.3	0.9	-0.4
CA 2	0.1	-0.4	0.9

3 **REFERENCE** Inverse Covariance Matrix

	%Chir	CA 1	CA 2
%Chir	1.39	-0.67	-0.44
CA 1	-0.67	1.66	0.77
CA 2	-0.44	0.77	1.48

4 Standarized Data for **DICKIE TEST SITE**

%Chir	CA 1	CA 2
-0.70	-1.04	-3.60

%Chir	-0.70
CA 1	-1.04
CA 2	-3.60

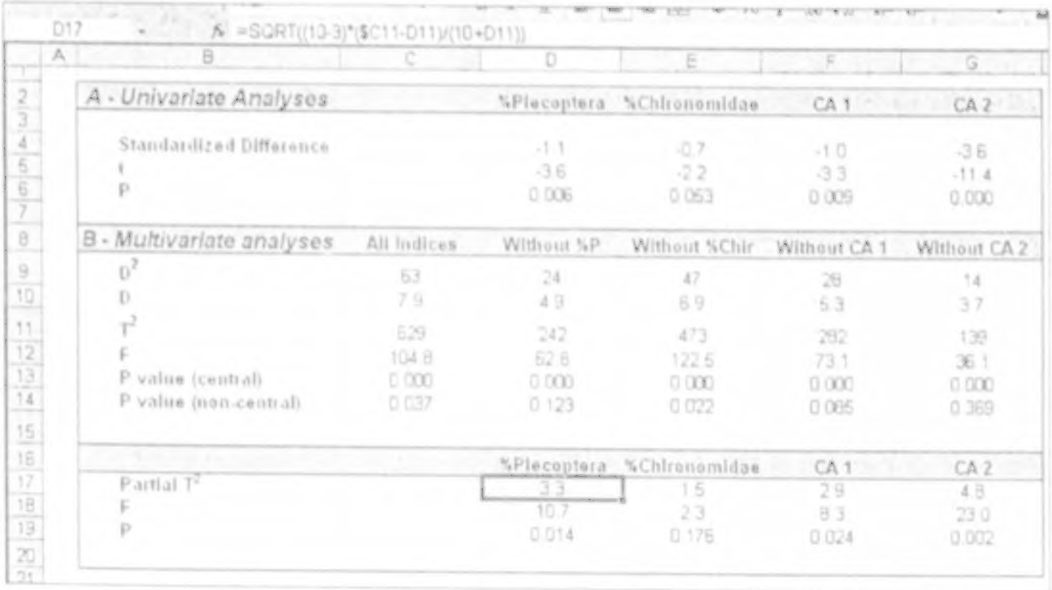
5 Product of Standarized Data and Inverse Matrices

	%Chir	CA 1	CA 2
DICKIE	1.30	-4.03	-5.83

6 Second Product using Transposed Test Data

	D^2	D	F	Lambda	p
DICKIE	24.2	4.9	63	78	0.123

Fig. 7. Initial six steps used to calculate partial T^2 for % Plecoptera metric (Chir = Chironomidae, CA 1 = scores on correspondence axes 1).



D17 =SQRT((10-3)*($C11-D11)/(10+D11))

A - Univariate Analyses		%Plecoptera	%Chironomidae	CA 1	CA 2
Standardized Difference		-1.1	-0.7	-1.0	-3.6
t		-3.6	-2.2	-3.3	-11.4
P		0.006	0.053	0.009	0.000
B - Multivariate analyses	All Indices	Without %P	Without %Chir	Without CA 1	Without CA 2
D^2	63	24	47	28	14
D	7.9	4.9	6.9	5.3	3.7
T^2	529	242	473	282	139
F	104.8	62.6	122.5	73.1	36.1
P value (central)	0.000	0.000	0.000	0.000	0.000
P value (non-central)	0.037	0.123	0.022	0.065	0.369
		%Plecoptera	%Chironomidae	CA 1	CA 2
Partial T^2		3.3	1.5	2.9	4.8
F		10.7	2.3	8.3	23.0
P		0.014	0.176	0.024	0.002

Fig. 8. Summary of TSA results (P = Plecoptera, Chir = Chironomidae, CA 1 = scores on correspondence axes 1).

Results and Discussion

The first 2 axes of the CA ordination accounted for 55% of the variation in the P/A data matrix (Fig. 2). Most of the reference sites clustered together in the ordination (Fig. 4), although the Britannia stream was separated from the others along the first CA axis because of the presence of zygopteran nymphs and the absence of anisopterans, tabanids and oligochaetes that were found in all other reference streams. The relative proportions of plecopterans and chironomids in the reference streams averaged 18 and 29%, respectively. By contrast, both insect groups were less frequent in the test stream, with <1% plecopterans and 18% chironomids. When expressed as differences from the reference-stream mean in units of reference-stream standard deviations, the % plecopterans was -1.1, the % chironomids was -0.70, CA 1 was -1.0, and CA 2 was 3.6 (Fig. 8A). This summary indicated that the test stream was most different from the reference streams on CA 2 (**t**=-11.375, **P**<0.001) This difference is likely due to the occurrence of isopods, megalopterans and amphipods in the test stream. No reference streams supported isopods, and relatively few reference streams had megalopterans (2) or amphipods (3).

Using the 4 metrics and the TSA method, we found that the benthic community in the test stream was significantly different from reference-stream communities (**D**=7.9, $\mathbf{T}^2$=629, **F**=104.8, **P** <0.001; Fig. 8). Moreover, the test stream was significantly outside the normal range of reference streams (non-central test, P=0.037). Based on the partial T^2 values, CA 2 added the most unique information (partial $\mathbf{T}^2$=4.8, **F**=23.0, **P**=0.002), whereas the % Chironomidae metric failed to add a significant amount of unique information to the multivariate analysis (*partial* $\mathbf{T}^2$=1.5, **F**=2.3, **P**=0.176). The test stream was most different from reference streams on CA 2 (highest univariate **t**, **P**<0.001), and the CA 2 metric contained the most unique information relative to the other 3 indices (highest partial $\mathbf{T}^2$, **P**=0.002). However, P values for the univariate t tests (e.g., P<0.001 for CA 2) were smaller than P values for the *partial* $\mathbf{T}^2$ tests (e.g., **P**=0.002 for CA 2), suggesting there was some correlation or redundant information among the indices we used. Omitting any one of the 4 indices from the multivariate $\mathbf{T}^2$ test did not change our conclusion that the test stream was significantly different from reference streams (i.e., D≠0); however, 3 of the 4 non-central tests (without %P, CA 1 and CA 2) failed to indicate that the test stream was outside of the normal range for reference streams when any one index was removed (i.e., **D**⇒normal range, **P**>0.05). This result underscores the importance of our choice of summary indices in benthic community assessments and highlights the fact that our statistical power will depend on that choice.

This simple demonstration illustrates that the TSA approach is an objective way to assess whether a test site differs from a set of reference sites. The resultant **P** value based on the multivariate $\mathbf{T}^2$ provides a single probability to evaluate a test site using a suite of summary biological indices simultaneously. Redundancies or correlations among the indices are factored out of the assessment by using the generalized distance (**D**). The non-central test evaluates the degree of impairment relative to a benchmark derived from the normal range of variation in the reference sites. Because the magnitude of **D** depends, in part, on the number of indices used in the assessment, we suggest using the **P** values associated with the tests as a means of comparing different test site analyses. We believe that the TSA method provides a relatively easy way to assess and interpret the degree of impairment at a test site. We predict this approach will also allow us to: (i) set critical effect sizes to suit the objectives of particular study design or management practice, (ii) test existing knowledge about the response of benthic invertebrates to anthropogenic stressors, and (iii), improve monitoring and rehabilitation endeavours by clearly identifying significant differences between test and reference sites.

Summary

To illustrate the TSA approach, we compared the benthic macroinvertebrate community from a test stream that was historically impacted by acid precipitation with benthic communities from a set of minimally impacted reference streams. Using calculations in a simple spreadsheet, we evaluated the biological condition of the test stream based on a number of summary biological indices, both individually and simultaneously. We also illustrated how to evaluate the contribution of each summary index to the assessment. Our use of a variety of summary indices to obtain a single statistical test of significance within the context of the reference-condition approach provides a simple and unambiguous framework for evaluating the biological condition of a test site.

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Defining reference and degraded conditions: integrating approaches to evaluating environment effects. J.J.H. Ciborowski[1], J.A. Schuldt[2], L.B. Johnson[3], G.E. Host[3], T. Hollenhorst[3] and C. Richards[4]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; [2]Department of Biological Sciences, University of Wisconsin-Superior, Superior, WI; [3]Natural Resources Research Institute, University of Minnesota-Duluth, Duluth, MN; and [4]Minnesota Sea Grant and College Program, University of Minnesota-Duluth, Duluth, MN.

Various models provide conceptual frameworks to classify areas and assess multiple effects of human activity on environmental condition. We show that locations can be ordinated along independent hypothetical axes representing different anthropogenic disturbances (SOLEC "pressures"). Axes diverge from a common hyperdimensional point, representing the absence of all pressures (pristine/control conditions). The diverging axes form edges of a pyramid, the base of which represents different types of "degraded condition." The Reference Condition is the suite of locations within a hypergeometric volume extending from the common point (apex) to locations along each axis at which the biotic community is meaningfully different than the community at the apex. The spatial correlation (r) between values of two different classes of pressure among locations defines the angle of divergence of the axes (arc-cos[r]), hence pyramid shape. The volume occupied by the Reference Condition relative to the whole pyramid quantifies "ecosystem health." Viewing the pyramid from the base gives a "pressure rosette" whose shape reflects the spatial autocorrelation among pressure classes. A "pressure pyramid" of Great Lakes coastal habitats is demonstrated using 5 GIS-based measures of human activity - agricultural land (%), urban land (%), road density, distance from an NPDES point source, population density. The method is amenable to evaluation of areas contaminated by chemical mixtures.

Using criteria in a biological monitoring and assessment framework. B.W. Kilgour[1], K.R. Munkittrick[2], C. Portt[3], K. Hedley[4], J.M. Culp[5], S.S. Dixit[6] and G. Pastershank[4]. [1]Jacques Whitford Environment Ltd., Ottawa, ON; [2]Department of Biology, University of New Brunswick, St. John, NB; [3]C. Portt and Associates, Guelph, ON; [4]Environment Canada, Environmental Quality Branch, Gatineau, QC; [5]Environment Canada, National Water Research Institute, Fredricton, NB; and [6]Environment Canada, National Guidelines and Standard Office, Gatineau, QC.

We designed a framework that integrates biological criteria for sentinel fish species, benthic communities and primary producers. These indicators were selected because their responses are predictive of effects on fish communities. For each indicator, a set of criteria was developed that determine the management reaction. Warning-level criteria for benthic communities are effects on indices of composition that exceed the mean reference response by ±2 standard deviations. For sentinel fish populations, warning-level effects are >25% changes in gonad or liver size, growth, or age, or >10% change in condition factor. For primary producers, warning-level effects are those that coincide with anticipated changes in fish populations based on existing models. Where warning-level criteria are exceeded, it is recommended that monitoring be repeated at two- to three-year intervals.

Where continued monitoring demonstrates an increase in the extent or magnitude of effects on benthic communities, or sentinel fish populations, it is recommended that effects be considered unacceptable and that the cause of effects be identified and managed. Losses of non-rare species or shifts in dominance are considered severe fish-community effects that would trigger management. Domination of the benthic community by one or a few tolerant taxa normally coincides with effects on fish communities, and should also be considered a severe effect that triggers management. The framework was designed with the intention that it could be applied to any point- or non-point discharge.

Deciding how to monitor marine oil spills: implementing environmental effects monitoring during oil spills in Canada. K. Trudel. S.L. Ross Environmental Research Ltd., Ottawa, ON.
This paper describes a three-tiered system for conducting environmental effects monitoring (EEM) for marine oil spills. Marine oil spills can be large and destructive, but most are small, causing little damage. When spills occur, EEM is needed for a variety of purposes, but can be costly. A plan has been developed to address the unique EEM needs of marine oil spills on an effective and cost-effective basis. This paper describes the unique needs for EEM for oil spills, the principles of EEM for marine spills and the implementation system matching level of effort (and cost) to the level of environmental damage caused. The plan addresses EEM needs for human health protection and environmental damage assessment. EEM for each target group involves numerous tasks. The plan divides the tasks for each target group into three tiers, each successive tier gathering more data and more reliable data than the one before. Monitoring effort is linked to damage or to predictors of spill damage (e.g., spill size of spill, oil persistence). Monitoring objectives, criteria/standards for escalating from one monitoring tier to the next are discussed. The method is based on experience in historical spills, but focuses on the industrial and environmental conditions of Canada's eastern coast. Linkages to operational EEM for offshore drilling are also discussed.

Shortcomings of using provincial environmental quality guidelines in risk assessment of polycyclic aromatic hydrocarbons. G. Gilron and A. Safruk. Cantox Environmental Inc., Mississauga, ON.
Polycyclic Aromatic Hydrocarbons (PAHs), a group of approximately 100 compounds composed of two or more fused benzene rings, are released from a variety of natural and anthropogenic sources and can reach elevated concentrations in areas of intense industrial activity. Concentrations of PAHs in the Great Lakes basin can contribute to degradation in aquatic ecosystem health. In Ontario, the Provincial Sediment Quality Guidelines and the Provincial Water Quality Objectives are used to protect aquatic biota from deleterious effects of contaminants, including PAHs. Specific sediment and water quality guideline/objective values for PAHs are often inconsistent or inappropriate, either by being set unreasonably low, or at levels that are not protective of aquatic organisms. When assessing risk from PAHs in sediment, using the provincial guidelines/objectives as benchmarks, it is often difficult to choose an appropriate benchmark value; for example, the Lowest Effect Levels (LELs) are often too conservative, while the Severe Effect Levels (SELs) are generally much higher than appropriate. The water quality objectives for some PAHs are set at unrealistically low levels considering the level of inputs to the environment; they are often set at levels that fail to approach even the lowest toxicological benchmark and in so doing, serve only to create scenarios which fail to meet objectives. We will discuss this issue using a comparative assessment of guidelines, toxicity data, and environmental concentrations.

Forensic ecotoxicology – a tool for managing toxics in the environment: gas plant effluent study. D.A. Birkholz[1], J.S. Goudey[2], M. Ralitsch[3], J. Proczkowski[1] and R. Brockbank[1]. [1]Enviro-Test

Laboratories, Edmonton, AB; HydroQual Laboratories Ltd., Calgary, AB; and [3]Enviro-Test Laboratories, Ottawa, ON.

In December of 2002, an effluent sample, submitted by a gas plant for routine testing, was observed to be toxic to trout. The company in question, responded immediately by initiating a toxicity identification evaluation. Solid phase extraction was successful in removing the toxic chemicals. Elution of the solid phase extraction cartridges with water: methanol solutions, followed by additional toxicity testing revealed that the toxic chemical were recovered at ambient and acidic pH by elution with 75% methanol in water. Analysis of the 75% methanol fraction revealed the presence of mono-, di- and tri-brominated tolyl triazoles. These were formed by the reaction of corrosion inhibitor(tolyl triazole) with oxidants (hypobromous acid) which are commonly used in cooling towers. The confirmation of the toxic chemicals was achieved by simulating the cooling tower operation in the laboratory. We were able to produce and isolate the toxic chemicals which were again identified as brominated tolyl trialzoles.

The fate, behavior, and ecotoxicity of new polymers in the aquatic environment. S. Lawuyi, S. Falicki, A.J. Atkinson and G. Hammond. Environment Canada, Gatineau, QC.

Over 6000 new polymers have been notified in Canada since the New Substances Notification Regulations of the *Canadian Environmental Protection Act*, was adopted. Technologically, advancement has led to increasing use of polymers as wood and steel substitutes that require special properties such as tensile strength, hardness, density variation, brittleness, surface resistance to abrasion and corrosion, and durability. They are also widely used as surfactants, coating agents, and adhesives. For some of these polymers, sound and accurate characterization of their properties relevant to an environmental risk assessment remains a challenge. Some of these properties include ecotoxicity, persistence, bioaccumulation, environmental fate and behaviour. The main focus of this talk will be on the fate, behaviour and the ecotoxicity of these new polymers to aquatic organisms.

Comparative toxicology of four crude oils. L.M. Clarke[1], P.V. Hodson[1] and R.S. Brown[2]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Department of Chemistry, Queen's University, Kingston, ON.

The toxicity of crude oil to the early life stages of fish has been linked to the presence and concentration of polycyclic aromatic hydrocarbons (PAHs). Fish that are chronically exposed to PAHs exhibit dioxin-like toxicity characterized by the presence of blue sac disease (BSD) and the induction of cytochrome P4501A (CYP1A). We compared the relative toxicity of four crude oils, Scotian Shelf (SS), MESA, the synthetic Alberta Sweet Mixed Blend (ASMB), and Alaska North Slope Crude (ANSC), for causing BSD by exposing rainbow trout (*Oncorhyncus mykiss*) embryos in simulated spawning beds containing gravel contaminated with one of four oils. Each oil had different chemical characteristics and PAH compositions, and our hypothesis was that the ASMB oil would be the most toxic due to its greater PAH concentration. The larvae were sampled at swim up for immunohistochemical staining, to characterize PAH exposure, and scoring for symptoms of BSD to characterize toxicity. Preliminary data indicates that the exposure of trout to PAH is greatest for ASMB oil, which is consistent with its higher concentration of PAH. These results indicate that it is the presence, concentration, and conformation of specific PAHs which is the primary cause of toxicity.

Temporal changes in contaminants and trophic levels in biota from Yukon subarctic lakes. M.J. Ryan[1], G.A. Stern[1], K.A. Kidd[1], M. Diamond[2], M.V. Croft[1], S. Gewurtz[2] and P. Roach[3]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]University of Toronto, Toronto, ON;

and [3]Department of Indian and Northern Affairs, Whitehorse, YT.
This research is aimed at evaluating temporal and spatial trends of Hg and organochlorines in the ecosystem of several Yukon lakes with a focus on Lake Laberge. Biota were sampled and frozen until analysed for stable isotopes and contaminants. Although there was a significant 30% decline in total Hg concentrations (1993-1996) from Laberge lake trout, there is no significant change from 1996-1998. A 50% decline in Hg concentrations was measured in trout from Kusawa Lake after 1999. A smaller decrease was observed in Quiet Lake trout between 1993-1999. There is little evidence to support any further changes in Hg tissue concentrations. OC concentrations in trout from Kusawa Lake have decreased over the 3-year period from 1999-2002. No apparent changes in OC levels in lake trout from Quiet Lake were observed and OC levels are significantly lower than those in trout from Kusawa and Laberge. Laberge lake trout have displayed a significant downward trend in OC over an 8 year period (1993-2001). The large downward trend in Laberge trout OC levels may be attributed to increases in overall fish populations offsetting ecosystem parameters such as trophic status of prey. Trophic levels and food sources as measured by stable N and C isotopes have shown that some species, such as northern pike, are shifting in the Laberge food chain. Some other species, such as snails, may have switched food sources or demonstrate a change in primary productivity of food sources based on C isotope data.

Revisiting the 1994 St Clair River sediment quality triad. T. Kierstead[1], T. Moran[1], M. Gardner[1] and S. Munro[2]. [1]Pollutech Enviroquatics Ltd., Point Edward, ON; and [2]Sarnia-Lambton Environmental Association, Sarnia, ON.

In the 1950's, extensive environmental monitoring of the St. Clair River, demonstrated a degraded environment characterized by poor water and sediment quality along the Ontario side of the river. Reports at the time attributed degradation to discharges from the extensive refining and petrochemical facilities in the area. Trends over time, from those early studies to recent studies, indicate that both water and sediment quality have improved. Using an integrated sediment assessment (Triad) approach, SLEA2 conducted a study of the industrial area in the Sarnia region of the St. Clair River in 1994-1996. The 1994 field work identified seven of 28 sampling sites within the three remaining impaired areas as having "strong evidence of pollution-induced degradation from sediments."

The SLEA library contains in excess of 100 reference documents and studies about St. Clair River water quality. These were reviewed with the intent of strengthening future testing methods by looking for past trends in approaches, methods and findings. The 2003 Triad study: focuses on monitoring locations at three "priority 1" zones identified in the 1994 work, coincides with bioaccumulation study locations that were established in 2001; and, includes upstream and far downstream control points. The Triad assessment and bioaccumulation studies form two components of a long term monitoring strategy, designed to establish trends in the quality of St. Clair River sediments adjacent to the most industrialized areas of the river. Of particular note, is the importance of considering tissue residues as part of the long term strategy. The results of this review are summarized and presented. The 2003 "Triad" study currently underway has had its design strengthened by adding 8 American reference locations included for the benthic macroinvertebrate assessment. The components of this revisit include: (i) benthic macroinvertebrate assessment, including specific habitat descriptors; (ii) a sediment toxicity assessment including *in-situ* 21 d fathead minnow survival and reproduction, tissue archival, and laboratory midge and amphipod survival and growth testing; and (iii) sediment quality (chemistry) assessment. The intent of the long-term study is to repeat the bioaccumulation and "triad" assessments at the established locations on a continuing three-year cycle, developing temporal trends using consistent data sets.

Evaluation and refinement of Environment Canada's primary hepatocyte test method for measuring acute lethality of metal mining effluents. R. Chong-kit[1], J. Schroeder[1], F. Gagné[2], D.G. Poirier[1], J.A. Miller[3] and R.P. Scroggins[4]. [1]Ontario Ministry of the Environment, Etobicoke, ON; [2]Environnement Canada, Centre Saint-Laurent, Montréal, Qc; [3]Miller Environmental Services Inc., Innisfil, ON; and [4]Environment Canada, Methods Development and Applications Section, Ottawa, ON. The Ontario Ministry of the Environment, in collaboration with Environment Canada's Methods Development and Applications Section and Centre St. Laurent, undertook an evaluation of Environment Canada's 1999 Toxicity Evaluation using rainbow trout hepatocytes. Tests were conducted on Cu and ammonia spiked into mining effluents and test medium and compared with toxicity to whole rainbow trout. Results of the tests indicated that the hepatocyte method was not sufficiently sensitive to contaminants commonly associated with metal mining effluents. Efforts to increase the sensitivity of the test included changes in the test medium, increasing the toxicant to cell ratio, and increasing test duration. However, no change resulted in sensitivity matching that of whole rainbow trout. In spite of the relatively low sensitivity of the hepatocytes to metals, refinement to the method improved precision of the test as illustrated through reference toxicant testing using KCl.

ETOX - database for aquatic and terrestrial ecotoxicological information. D. Schudoma. Federal Environmental Agency, Berlin, Germany.
ETOX is a new database for aquatic and terrestrial ecotoxicological information as well as for guideline and standard values developed by the German Federal Environmental Agency (Umweltbundesamt). This system is composed of an efficient MS SQL-server database, which can be accessed via a MS Access 2000-client for administrative purposes in the Federal Environmental Agency. For externally data retrievals a web-application (http://anubis.uba.de) was developed. The database ETOX 1.0 contains 22000 ecotoxicological data and around 3000 data of international quality objectives. A further development of the prototypical the web-application is planned. A advanced search and data input via internet will be developed. The poster will present the database structure and functions with the aim to discuss the cooperation with other institutions working with similar projects.

The changing face of aquatic toxicology in the Ontario Provincial Government. D.G. Poirier, J. Schroeder, R. Chong-kit, T.L. Watson-Leung, M. Mueller, M. Appleton and A. Collins. Ontario Ministry of the Environment, Etobicoke, ON.
In 1985, Ontario was using a single test method (the 96 h static acute lethality test with rainbow trout, *Oncorhynchus mykiss*), to measure potential environmental impacts of a few major industrial sectors. Since that time there have been significant changes in the way the Ontario provincial government practices the science of aquatic toxicology. In 1988 the rainbow trout test was joined by an acute lethality test with *Daphnia* just in time for the monitoring phase of MISA. The transition from monitoring to effluent limits regulations in 1995 resulted in a significant reduction in the number of acutely lethal effluents, and allowed us to change our focus to assisting regional abatement staff and industries with site specific toxicity concerns. This customer service orientation has carried through into our formation of partnerships with Environment Canada, municipalities, other Ministries and MOE divisions, and has culminated in our accreditation under the SCC/CAEAL. Presently we are supporting an increase in enforcement and contaminated site assessment activities by MOE and Environment Canada regional staff. Applied research is occupying more of our time as our laboratory analyses data and develops methods to address concerns with Animals for Research (using screening tests), human health (West Nile Virus control), potential sublethal impacts, new product usage (MTO), PWQO development, and new effluent treatment technologies (Municipality of Toronto). Results of

tests conducted for the MISA program over the past several years, as well as selected case studies will be presented to illustrate the success of effluent compliance strategies and our changing focus.

UV-B radiation and 4-tert-octylphenol reduce survival and induce developmental abnormalities in *Rana pipiens* **tadpoles**. M.C. Croteau, D. Lean and V.L. Trudeau. Department of Biology, University of Ottawa, Ottawa, ON.

Endocrine disrupting chemicals such as 4-tert-octylphenol (4-t-OP) are found in many aquatic systems and are capable of disrupting the development and metamorphosis of amphibians. UV-B radiation can cause severe developmental abnormalities and can be detrimental to survival. Our study examines the effects of possible synergistic interactions between UV-B radiation and 4-t-OP on amphibian development and survival. Tadpoles (*Rana pipiens*) were exposed to one of two concentrations of 4-t-OP (0.206 μg/L, 206 μg/L) plus a 0.01% ethanol solvent control and a clean water control, with and without UV-B radiation (mean intensity of 22 μW/cm2). After 8 months of exposure, the animals were transferred to a clean water grow-out system until metamorphosis. Results indicate that tadpoles exposed to a combination of UV-B + 4-t-OP have the highest incidence of mortality. After only one week of exposure, the mortality of animals in these groups was considerably higher (22-30%) when compared to the control treatments (6-9%). A similar trend was also observed for the incidence of developmental abnormalities and malformations. After 48 weeks of exposure, tadpoles in the UV-B + 0.206 μg/L 4-t-OP treatment were affected by twice as many deformities (59%) than those in the control groups (27-33%). Spinal curvature, abdominal edema and abdominal abnormalities were the main developmental abnormalities and malformations observed in this study. These results show that interactions exist between UV-B and 4-t-OP to affect development and survival in developing tadpoles. This work was supported by CNTC, NSERC and OGS.

Acute toxicity of the mosquito-control pesticide, malathion to northern leopard frog (*Rana pipiens*) **tadpoles**. B. Perez[1], N. Gallant[2], J. Gibson[2], B.D. Pauli[3], D. Lean[4] and V.L. Trudeau[4]. [1]Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, ON; [2]University of Ottawa, Ottawa, ON; [3]Environment Canada, National Wildlife Research Centre, Gatineau, QC; [4]Department of Biology, University of Ottawa, Ottawa, ON.

Malathion, an organophosphate insecticide with a wide variety of uses in agriculture, can also be applied near wetlands and in recreational areas for control of adult mosquitos. Non-target organisms may also be affected by malathion. Preliminary experiments demonstrated that a 4 d exposure to malathion resulted in 100% mortality of tadpoles at concentrations that were 100,000 times lower than the suggested dilution by the manufacturer. We exposed tadpoles at post-hatch (Gosner stage 25) to five concentrations of a domestic formulation of malathion (50%) ranging from 10 mg/L to 100 μg/L. One exposure series was conducted under normal full spectrum fluorescent lighting while the other series was exposed under UV-B light at an intensity of 22 μW/cm2 (approx. UV-B levels found in surface water). The exposures were conducted over eight days and water was renewed daily. The LC50 was calculated using a trimmed Spearman Karber method. The LC50 for non-UV exposed tadpoles was 7.1 μl/L while the LC50 for UV exposed tadpoles was three times lower at 2.3 μl/L. Swimming ability was impaired in both UV and non-UV treatments when compared to the control group. These results demonstrate that environmentally relevant levels of UV radiation can increase the acute toxicity of malathion to larval amphibians. Supported by NSERC and CNTC.

Acute and sublethal toxicity studies of rotenone in rainbow trout (*Oncorhynchus mykiss*). W.W.L. Cheng and A.P. Farrell. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

Abstract

Rotenone, a piscicide, was shown to have an extremely small margin (1.6 μg/L) between no lethalithy (5 μg/L) and 100% mortality (6.6 μg/L) for the 96 h tests (LC50=5.8 μg/L-) in juvenile rainbow trout (*Oncorhynchus mykiss*). Hydrophobic rotenone adsorbed onto dissolved organic carbon (DOC) at 3.0 and 4.0 mg/L, significantly reduced acute toxicity in a dose-dependent manner. LC50 was increased from 5.80 (C.L.s 5.51-6.10) to 6.55 (C.L.s 6.28-6.83) and 7.75 (C.L.s 7.29-8.24) μg/L, respectively. Rotenone adsorption onto DOC may have decreased its bioavailability, thus reducing the acute toxicity. The sublethal effect of rotenone on critical swimming performance (Ucrit) of rainbow trout was investigated. Fish were exposed to 0, 3.0, 4.0, and 5.0 μg/L of rotenone for 2, 4, 6, 12, 16, 24 and 48 h exposure periods and tested using a ramp velocity testing protocol. Rotenone demonstrated an "all-or-none" concentration effect (p=0.029) on mean Ucrit at ≥3.0 μg/L. No evidence was found for either interaction between effects of exposure time and rotenone concentrations on mean Ucrit, or a time-dependent effect on mean Ucrit.

Oxygen consumption rate (Mo2) of rainbow trout exposed to 0, 1.5, 2.5, 3.0 and 3.5 μg/L of rotenone was monitored using continuous respirometry for 96 h. Comparisons were made between pre-exposure (0-48 h) and exposure period (48-96 h), and also with control fish. Rotenone exhibited an "all-or-none" reduction on peak active metabolic rate (Peak AMR) at 1.5-3.5 μg/L but showed no effect on routine Mo2 (RMR). Exercised fish were individually monitored for initial post-exercise oxygen consumption rate (Mo2Max, 5 min) and excess post-exercise oxygen consumption (EPOC40 min) at 0, 1.0, 3.0, 4.0, 5.0 and 6.0 μg/L rotenone levels. Mo2Max and EPOC values were compared before and after treatment, and with the control. Rotenone significantly increased the differences in Mo2Max and total EPOC (before and after treatment) comparing to the control, at concentrations of 4.0 and 5.0, but not at 6.0 μg/L, which approximates the LC50 value. Rotenone showed characteristics of "all-or-none" toxicity to rainbow trout, having an extremely narrow margin for effects. Thus, it is not recommended for selective killing. Further, judicious action is needed in rotenone application for total reclamation to protect non-target species.

Introduction

Our objectives are three fold. First, is to study the effects of DOC from both natural and commercial sources on the acute toxicity of hydrophobic rotenone (Gilderhus et al. 1988). Second, is to determine the acute toxicity of rotenone (96 h LC50), and sublethal effects on Ucrit (Jain et al. 1997), routine metabolic rate (Mo2) (Brett 1964, Skadsen et al. 1980), Mo2Max and EPOC (Lee et al. 2003). Third is to enhance the knowledge on rotenone acute and sublethal toxicities in trout, as it is in the process of re-registration in Canada and the United States.

Materials and Methods

Juvenile rainbow trout, *Oncorhynchus mykiss*,(2.0-4.0 g) were obtained from local trout farm (Mission, BC) were acclimated under control conditions for 1 week in 750 L fibreglass holding tanks at a density of ca 0.5 g/L. They were held under a 12 h light: 12 h dark photoperiod at 10-15°C in flowing dechlorinated municipal water at pH 6.86±0.10 with oxygen saturation of >95% and hardness of 6.73±0.07 mg/L CaCO3 (Environment Canada Laboratory), except fish for the respirometry test were held in photoperiod of 0 h L: 24 h D. They were fed with commercial starter feeder (Nutra Plus, Crumb #2) six times per week, but were deprived of food for 24 h prior to testing to assure a post-absorptive state.

Fraser River sediments were collected from the shoreline of Iona Beach during a low-tide period. They were dried at 60°C overnight and then sieved through 300 micron mesh. Rotenone (95-98%), N, N-dimethyl formamide, and humic acids were obtained from Aldrich Sigma Chemical Co. Stock solutions of rotenone at 2.0 g/L were prepared daily for each experimental trial by dissolving 0.01 g of rotenone in 5 ml of dimethylformamide. Static renewal 96 h LC50 tests were performed with and without DOC in the forms of 0.2% B.C Fraser River sediments (<300 mm), and commercial humic acids.

Ucrit was measured using a Blazka-type swim chamber and a ramp velocity testing protocol (n=6-10). Fish were exposed to 0, 3.0, 4.0 and 5.0 μg/L of rotenone for 2, 4, 6, 12, 16, 24 and 48 h exposure periods before transferring to the swim chamber for swim performance test.

Mo2 was measured using a computer-controlled, intermittent-flow respirometer (48 h pre-exposure: 48 h exposure); Peak AMR and RMR were derived from exposure period (n=4-8).

In Mo2Max and EPOC tests (n=5), fish were individually chased, and Mo2 was monitored for 40 min in dechlorinated water (control, before treatment); fish were then chased and transferred to the toxicant and the Mo2 re-measured (exposed, after treatment); comparisons were made for Mo2Max during the first 5 min after chasing and for EPOC40 min.

Results

Static renewal 96 h LC50 of rotenone in juvenile rainbow trout was 5.8 μg/L with 0% mortality at 5.0 μg/L and 100% at 6.6 μg/L. DOC in the form of humic acids increased LC50 at 3.0 and 4.0 mg/L, but not at 2.0 mg/L. Rotenone toxicity decreased by about 50% at DOC of 4 mg/L (Fig. 1). An "all-or-none" sub-lethal effect with significant decreases in Ucrit (p=0.029) by about 10% at $\geq$3.0 μg/L was observed (Fig. 2); however, there was no evidence (p=0.997) from interaction between the effects of exposure periods and rotenone concentrations upon Ucrit , or effects of exposure on Ucrit. An "all-or-none" effect with significant decreases in Peak AMR (p=0.022) by about 30% at $\geq$1.5 μg/L (Fig. 3) was observed. Peak AMR recured at intervals of ca 24 h measured at around 03:00 h. Mean differences of Mo2Max (p=0.003) and total EPOC (p=0.001) before and after treatments were significantly increased at 4.0 and 5.0 μg/L comparing to the control (Figs. 4 and 5), but showed no effect at 6.0 μg/L, thus, they are not dose dependent.

Conclusions

Rotenone was found to demonstrate a characteristic "all-or-none" toxicity to rainbow trout as shown in both Ucrit test and Peak AMR, and an extremely small margin between no lethality and 100% mortality for 96 h tests. In addition, the presence of organic carbons, light, temperature, and air may confound the persistence and availability of rotenone in the natural environment. To control rotenone levels to below thresholds for effects in the wild would be a challenge. Thus, it is not recommended for selective killing. Judicious application will allow the continued use of rotenone in total reclamation to protect non-target organisms.

Acknowledgements

Funding for this project was provided by Environmental Conservation Branch, Environment Canada. Special thanks to Dr. Kurt Gamperl, Newfoundland Memorial University, in loaning the swim chamber for swimming performance test, and to the Chemistry Laboratory of Pacific Environmental Science Center, Environment Canada, in supplying chemistry analyses.

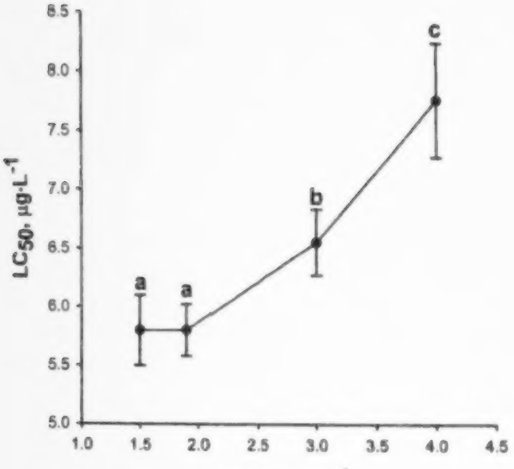

Fig. 1. Acute lethality of rotenone as a function of dissolved organic carbon (DOC, mg/L) in rainbow trout. Sources of DOC are Fraser River sediments (0.2%) and commercial humic acids (5 and 19 mg/L). Means not sharing a common letter were significantly different ($P<0.05$).

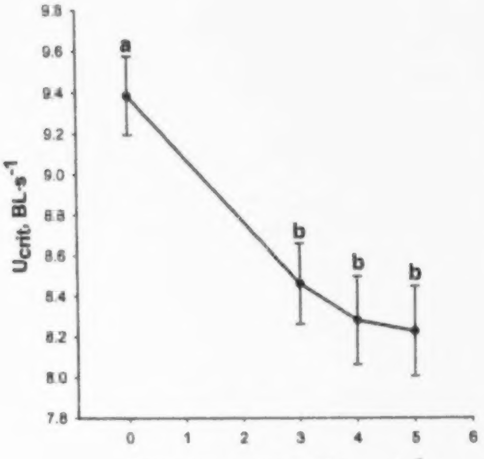

Fig. 2. Effect of rotenone concentrations of all exposure duration on critical swimming speed of juvenile rainbow tour (BL+5.9±9.1 cm, n=6). **a,b** levels not connected by the same letter are significantly different.

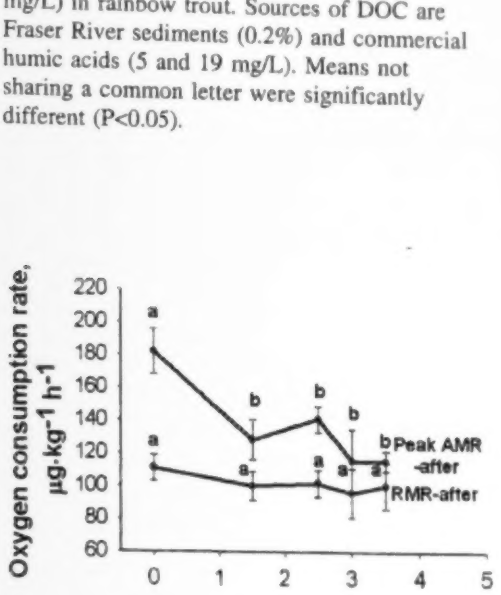

Fig. 3. Comparison of peak AMR and RMR of juvenile rainbow trout after exposure (48-96 h) to rotenone as a function of concentration.

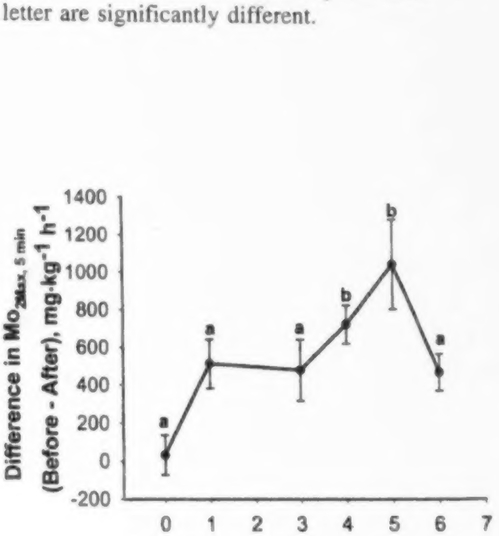

Fig. 4. Differences in initial metabolic rate post exhaustion ($Mo_{2Max,\ 5\ min}$) before and after rotenone exposure as a function of rotenone concentration in juvenile rainbow trout (2,7±0.1 g, n=5). **a,b** levels not connected by the same letter are significantly different.

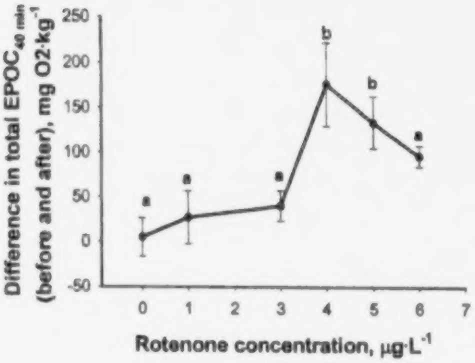

Fig. 5. Differences in total EPOC$_{40\ min}$ before and after rotenone exposures as a function of rotenone concentration in juvenile rainbow trout (2.7±0.1 g, n=5). **a,b** levels not connected by the same letter are significantly different.

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Substantiation validation of "low concern polymer" criteria for the assessment of new substances under the *Canadian Environmental Protection Act*. E. Karalis[1], A.J. Atkinson[1], G. Hammond[1], M.J. Lapointe[1] and T. Singer[2]. [1]Environment Canada, Gatineau, QC; and [2]Health Canada, Ottawa, ON.

The *New Substances Notification Regulations* (NSNR) of the *Canadian Environmental Protection Act* (CEPA(1999)) requires that new substances, including polymers, be notified to the Government and assessed for risks they may pose to the environment and human health. The Regulations contain criteria, based on those from the United States Environmental Protection Agency (US EPA), which are used to establish the regulatory definition of "low concern" polymers. By definition, low concern polymers have high number-average molecular weights, limited percentages of low molecular weight constituents, are chemically stable and have limitations on certain reactive and cationic moieties. These criteria have also been adopted by the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Polymers that meet the regulatory low concern criteria are believed to be of low inherent hazard to the environment and human health, and are subject to reduced information requirements. The purpose of this study is to gather data from ecotoxicity, mammalian toxicity and environmental fate studies for polymers assessed by Health Canada and Environment Canada which meet the low concern criteria to see if test results substantiate the criteria, thereby supporting the continued use of these criteria in reducing reporting requirements for certain polymers.

Draft follow up environmental risk assessment of chlorinated paraffins in Canada. L.S. Lander[1] and D.C.G. Muir[2]. [1]Environment Canada, Existing Substances Branch, Gatineau, QC; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

An environmental and health risk assessment of short, medium and long-chain chlorinated paraffins was published by the Government of Canada in 1993 under the Priority Substances Program. Short-chain chlorinated paraffins (SCCPs) were declared "toxic" to human health under Paragraph 11(c) of the *Canadian Environmental Protection Act* (CEPA) 1988. However, there was insufficient information to conclude if short, medium or long-chain chlorinated paraffins were toxic to the environment as defined under CEPA. In order to reconsider the determination of "toxic" under Paragraph 64(a) of CEPA 1999 for SCCPs and other chlorinated paraffins (CPs), as well as the persistence and bioaccumulation criteria, scientists at the National Water Research Institute of Environment Canada and the Department of Fisheries and Oceans have generated new data on levels of CPs in Canadian environmental media, including Arctic air, marine mammals, wastewater treatment effluents from southern Ontario, Canadian lake sediments, Lake Ontario fish and Lake Ontario water. This new information is being used to re-assess the environmental risk of chlorinated paraffins. The new data on levels of short, medium and long-chain chlorinated paraffins in the Canadian environment will be presented, as well as toxicity data, and data gaps. The results of the assessment work to date will be presented.

Ecosystem stress response: understanding effects on the benthic invertebrate community of Alberta oil-sands wetlands. C.M. Wytrykush and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

The purpose of this research is to examine environmental stress response of invertebrates using wetlands in the Alberta oil-sands region as a model. Wetlands in this area are either naturally occurring, or have been affected by oil-sands mine process materials (mine-tailings and/or saline process water). Oil-sands mine process materials are enriched with sulphate ions, ammonia, polycyclic aromatic hydrocarbons (PAHs), and naphthenic acids, which in high concentrations are potentially toxic to aquatic invertebrates, and are considered here as environmental stress. These wetlands can be classified as young or mature and as having low or high sediment organic content. We investigating food web dynamics and structure in wetlands contrasting in age, organic carbon base and anthropogenic stress to test several hypotheses relating to the effects of stress on ecological communities. Food webs will be constructed for the benthic macroinvertebrate community in stressed and unstressed wetlands. Primary and secondary production in these wetlands and estimate invertebrate diversity will be measured This information will be used to quantify the relationship between productivity and diversity. Stable carbon and nitrogen isotopes will be used to determine maximum trophic position, which indicates food chain length. It is expected that food chain length will be influenced by energetic constraints, ecosystem size and ecosystem stressors. This research complements previous work concerning the aquatic macroinvertebrates in these wetlands at the individual and the population level. This project is part of a collaborative effort to investigate environmental pressures using the Alberta oil-sands region as a model. It quantifies the dynamics of the important link between the responses to environmental pressures in aquatic systems and effects on terrestrial communities.

Monitoring degradation of oil sands constituents and foodweb dynamics in aquatic reclamation using stable isotopes. A.J. Farwell[1], B.J. Butler[1], M.D. Mackinnon[2] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Syncrude Canada Ltd., Edmonton, AB.
The extraction of bitumen from the Athabasca oil sands deposit in northern Alberta, Canada generates large volumes of process-affected water containing acutely toxic constituents such as naphthenic acids. Naphthenic acids are biodegraded and become less toxic in reclaimed aquatic systems. Stable isotopes were used to examine the cycling of oil sands constituents in aquatic systems to better understand potential ecological effects. Benthic invertebrates were collected from a series of test pits (Syncrude Canada Ltd.) constructed in 1989 (size, 0.16 ha) that differ in the quantity of process-affected water and/or mature fine tailings (MFT) containing residual bitumen. Dragonflies and damselflies showed trends of ^{13}C depletion and ^{15}N enrichment in pits with increased levels of process-affected water and/or MFT whereas only ^{15}N enrichment was observed for other groups including chironomids and amphipods. The significant ^{13}C depletion (~-27%) in highly turbid waters with elevated process-affected water and/or MFT suggests invertebrate assimilation and incorporation of oil sands constituents via the microbial foodweb. Naphthenic acids, up to ~25 mg/L in test pits, may represent a significant carbon source in reclaimed systems. Therefore analyses of extracted naphthenic acids from a number of different sources/ages and laboratory biodegradation studies were used to further define the isotope pathway of naphthenic acid degradation.

Bioaccumulation of hexachlorobenzene in the aquatic food web. D. Schudoma. Federal Environmental Agency, Berlin, Germany.
Hexachlorobenzene (HCB) is classified as priority hazardous substance under the European Water Framework Directive and will be subject for the derivation of a Environmental Quality Standard. At many measuring stations of the Joint Water Commission of the Federal States (LAWA), it has proved that the HCB concentration in the water phase was below the detection limit. As HCB accumulates in suspended particulate matter (SPM), SPM-bearing sediments and aquatic organisms, the HCB concentrations in these media are usually far above the detection limit. Therefore, a Biota-SPM Accumulation Factor (BSAF) can be calculated for most sampling sites for which data on residual levels in fish and in SPM or SPM-bearing sediments are available. Data on residual levels of HCB in bream (*Abramis brama*) and eel (*Anguilla anguilla*) are available from investigations carried out for the Environmental Specimen Bank, the Joint Working Group Elbe (ARGE Elbe) and the International Commission for the Protection of the Rhine. Using these data a BSAF can be determined for muscle tissue of bream and eel. The use of a BSAF to calculate a SPM Quality Standard for HCB corresponding to a Quality Standard referring to the protection of top predators from secondary poisoning will be discussed.

Review of current BSAF determinations and application in environmental assessments. T.L. Watson-Leung and J. Schroeder. Ontario Ministry of the Environment, Etobicoke, ON.
In 1992, the Ontario Ministry of the Environment (MOE) produced a protocol for measuring the toxicity and bioaccumulation potential of sediment associated contaminants using fathead minnows (*Pimephales promelas*). Fish tissue are analysed for contaminants of concern at the end of the

exposure period and a biota sediment accumulation factor (BSAF) for contaminant(s) of interest is calculated using lipid-normalized tissue concentration divided by the organic carbon normalized sediment concentration. The procedures followed in the 1992 protocol are currently under review with the aim to update and revise the protocol over the next few years. As a first step, the Ministry reviewed published literature on the determination and application of BSAFs in environmental assessments and compared theoretical relationships to MOE data from whole-sediment toxicity studies. A summary of BSAF values and current and future applications for selected chemicals are presented with reference to data collected by the MOE over the last ten years.

Adverse effects on fauna of Ami River by the effluents released by the Gorakhpur Industrial Development Authority (India). R.K. Misra[1] and S. Misra[2]. [1]Department of Zoology, R.M.L.A. University, India; and [2]Department of Biology, York University, Toronto, ON.

Abstract

Ami River is situated at the Gorakhpur Industrial Development Authority (GIDA), U.P., India. There are industrial sources as well as farming fields that discharge waste water effluents and are sources of pollution affecting flora and fauna of the river. The samples were collected from ten sites to give a range of different physicochemical parameters and the level of common urban contaminants. The sample contaminants measured were, polycyclic aromatic hydrocarbons (PAH's), benzene, toluene, ethylbenzene, xylene (BTEX); petroleum hydrocarbons, organochlorine pesticides (OCP); and heavy metals. The ranges of total petroleum hydrocarbons were minimum 5.6 and maximum 980, total PAHs 0.05-17.96, total BTEX <0.05-0.45, and total OCP <0.065 - 0.26 mg/kg, respectively. Heavy metals occurred above detection limits at all sites and the concentration of Zn very high at some sites 2300 mg/Kg. However most organic compounds occurred below detection limits. The fauna samples for the study were collected from the species of mollusk fresh water mussel *Unio* sp, and fishes *Gadusia* sp, *Xenentodon* sp, *Eutropichthys* sp, *Amphipnous* sp, and H*emiramphus* sp. Contaminants enter the organisms by absorption through surface of the body, particularly respiratory tract and or by ingestion of food and particulates. OCP concentration in the liver can be two or three magnitudes higher than the muscle tissue. Environmental factors and the form of Hg modify the uptake rate of Hg.

Introduction

Contamination may be defined as the presence of a component foreign to or greatly in excess of the normal requirements of the animal. Urban development incatchments has a major impact on the water quality, which are exposed to a range of contaminants from diffuse, and point source discharges, such as industrial effluents and storm water runoff. Urban storm water can be contaminated with a wide range of materials including dust and soil, litter and garden rubbish, animal wastes , fertilizers and pesticides, oil and grease, petroleum Hydrocarbons, sewage overflows, and tip and landfill lachate (O'Loughlin at al. 1992). Over the last two decades methods for the assessments of river ecosystem health have focused on measuring the condition of one or more important components of the ecosystem, usually the fish or macroinvertibrate communities (Karr 1981, Plakfin et al. 1989, Wright 1995).

Chlorinated organic contaminants released to the environment are of wide concern for their toxicity, persistence in the environment, bioaccumulation through food chains, and the public health aspects(Mah et al. 1989, Rogers et al. 1989 and Whitehead et al. 1992). Benthic stream fauna (invertebrates) are in close contact to contaminated sediments, many species feed extensively on those sediments, and these species form the trophic basis for most fish production in river ecosystem, as a result of the above there is a need to consider the concentration of these contaminants in benthic food webs and variations in their concentration across different species. In the aquatic food web contaminated organisms may also be a potential food source for other organisms at higher trophic level and therefore an exposure source for them too. This paper provides a prospective on the present extent of the contamination of the Ami River fauna and some of the contaminants such as hydrocarbons, OCP, PAHs, BTEX, petroleum hydrocarbons and heavy metals.

Materials and Methods

Ami River is situated in the Gorakhpur Industrial Development Authority (Fig. 1). There are industrial sources as well as farming fields that discharge waste water effluents and are source of pollution, often maximized in very localized area of the river. The chemicals enter the Ami River directly from drain or from atmospheric volatization because of large-scale use of pesticides, herbicides and fungicides. Hg is released in the Ami River water from industrial activities including leather tanning, electroplating and chemical manufacturing. An indirect source of Hg in water is Hg in the air; it is deposited from rain and other processes.

Sample collection: The contaminants were selected on the basis of their likely occurrence above detection limits in Ami River and their known toxicity (Table 1). The samples of the faunas and sediments were collected between 15 March–15 April and 15 July–15 Aug to represent the lowest and highest flow of the water in the river. Species of fresh water teleost fishes namely *Gadusia* sp.,*Xenentodon* sp. *Eutropichthys* sp. *Amphipnous* sp. *Hemiramphus* sp. And the fresh water mussel clam *Unio* sp. were selected for the study purposes because they are easily cacheable and available in the river. Gill nets were used for the fishes and fresh water mussel (*Unio* sp.) was collected with a clam rake or small dredge. After collection fishes and mussels were wrapped in aluminum foil and placed in wet ice. Sampling was carried out at ten different sites and returned to the laboratory.

Table 1. Contaminant groups monitored and their probable sources

Group	Sources
Petroleum	Biogenic- Products of organisms including volatiles hydrocarbons
Pyrogenic	Products of combustion, eneral storm water, road runoff, vehicle services areas and municipal wastes
Petrogenic	Petroleum products, industrial effluents
PAHs	Industrial effluents, timber yards, atmospheric inputs from settled particulate air pollution, runoff from roofings ,car service areas and road runoffs (Pitt et al. 1995)
BTEX	Organic solvent oils used in industries
OC pesticides	Drains from farming fields and storage areas
Heavy metals	Industrial effluents, car repairs, parking areas (Pitt et al. 1995)

Sediment for the determination of heavy metals was collected from depositional area at each site by scooping sediment into a plastic bucket, and sieved with thin nylon mesh and approximately 200 gm of the sieved material was retained in glass jars for laboratory analysis. Stainless steel container was used to scoop handful of material for the organics and also sieved and kept in glass jars and kept chilled until analysis. The extraction and analysis were done with the help of gas chromatography and mass spectrometry (GC/MS) methods (Benkert 1992).

Table 2. Min, mean and max concentrations in mg/kg of contaminants measured during the study. Mean values calculated by assuming the detection limit was the actual concentration of the contaminant recorded

Group/Contaminant	Minimum	Mean	Maximum
PAHs			
Napthelene	<0.05	<0.050	0.12
Fluorine	<0.05	<0.05	0.1
Anthracene	<0.05	0.15	0.42
Pyrene	<0.05	0.91	3.3
Benzo(αβγ) Pyrene	<0.05	0.59	3.1
Anthracene	<0.05	0.15	0.42
Total PAHs	<0.05	3.82	17.96
Benzene,Toluene	<0.05	0.07	0.039
Ethylbenzene,Toluene	<0.05	<0.05	0.15
Xylene(BTEX), Ethylbenz.	<0.05	<0.05	<0.05
Xylene	<0.05	<0.05	<0.05
Total BTEX	<0.05	0.25	0.45
Petroleum hydrocarbons			
C6-C9	5.9	7.01	8.89
C10-C14	5.98	16.01	28.9
C15-C28	4.00	75.02	630.0
C29-C36	6.12	58.98	560.0
Total	5.6	98.0	980.0
Organochlorines			
HCB	<0.05	0.009	0.009
Dicloran	<0.05	0.03	0.03
Total BHC(αβγ)	<0.05	0.06	0.020
Lindane	<0.05	0.006	0.006
Heptachlor	<0.05	0.06	0.019
Total Endosulphan	<0.05	0.030	0.056
Aldrin	<0.05	0.006	0.009
Dieldrin	<0.05	0.015	0.032
Endrin	<0.05	0.015	0.035
op-DDT,ppDDT	<0.65	0.042	0.16
Total OCs	<0.065	0.042	0.26
Total PCBs	<0.031	0.035	0.055
Metals			
Hg	<0.0035	0.21	2.1
Cd	0.025	0.49	5.98
Pb	3.14	65.89	5.69
Cu	0.05	29.45	230
Cr	3.1	45.36	263
As	0.96	7.96	78.0
Zn	21.65	301.12	2300

Table 3. Concentrations of petroleum hydrocarbon levels in Ami River fauna (mg/kg wet weight).

Fauna	Hydrocarbon	Conc.
Unio sp.	C14-C20	3.5
Gadusia sp.	Fuel oil	54
Xenentodon sp.	Fuel oil	64
Amphipnous sp.	C14-C20	4.00
Eutropychthys sp.	Benzopyrene	0.5
Hemiramphus sp.	C14-C20	0.3

Table 4. Range of pesticides and PCBs concentrations in the fauna of Ami River (μg/kg wet weight).

Fauna	Dieldrin	Total DDT	PCBs
Unio sp.	10-70	10-120	10-1700
Gadusia sp.	20-110	50-2200	30-3200
Xenentodon sp.	1-34	35-170	40-330
Eutropychthys sp.	1-23	3-52	1-99
Amphipnous sp.		390-1600	2200-8800
Hemiramphus sp.			5700

Results and Discussion

Samples from the Ami River, *Unio* sp., and some fishes were collected from ten different sites and analyzed for PAH's, BTEX, petroleum hydrocarbons, OCP and heavy metals. Most sample concentrations were either beneath the detection limit or too low to be associated with biological impacts.

There was some relationship between the concentrations of heavy metals and concentration of PAHs above detection limits the minimum PAH were<0.05 mg/kg and where maximum it was 17.96 mg/kg. Where there was highest level of PAH it appeared to be dependent of some factor of seasonality and due to continuous local point of contamination, the total BTEX was <0.05 mg/kg and maximum 0.45 mg/Kg, only benzene and toluene were recorded above detection limit thus BTEX does not appear to be a significant contamination in Ami River. Petroleum hydrocarbon appeared to be high concentration (980 mg/kg maximum and minimum 5.6 mg/kg) in both industrial sites and sites of urban rural fringe. Industrial sites are probably contaminated with industrial effluents and road runoffs, while the urban rural fringe sites may be contaminated with runoff from petrol stations and vehicle maintenance garages.

Table 5. Metals in the fauna in Ami river (mg/kg of weight). Values shown represent the number, mean± and range.

Fauna	Hg	Pb	Cd	Cr	As
Unio sp.	22	9	12	6	11
	0.08±0.05	1.60±2.28	0.22±0.17	0.33±0.12	3.13±3.85
	0.01-0.26	0.15-7.37	0.03-0.58	0.17-0.44	0.90-12.72
Gadusia sp.	27	3	3	5	5
	0.22±0.20	0.36±0.17	0.03±0.01	0.09±0.08	2.44±2.92
	0.22-0.80	0.20-0.53	0.03-0.04	0.01-0.18	0.11-0.51
Xenentodon sp.	9	3	3	5	2
	0.07±0.06	0.33±0.17	0.05±0.03	0.11±0.12	2.56±2.77
	0.02-0.17	0.20-0.53	0.03-0.08	0.02-0.30	0.60-4.51
Eutropychthys sp.	0	3	3	0	3
	0	0.28±0.07	0.04±0.02	0	2.4±1.26
	0	0.20-0.34	0.03-0.08	0	0.86-3.37
Amphipnous sp.	10	2	2	2	0
	0.24±0.13	0.44±0.10	0.04±0.03	0.09±0.00	0
	0.09-0.45	0.38-0.51	0.02-0.05	0.09-0.09	0
Hemiramphus sp	4	1	2	1	1
	0.12±0.08	1.64±1.70	0.26±0.29	0.08±0.10	2.35±2.39
	0.05-0.23		0.05-0.46		

OCP had a similar pattern of occurrence as petroleum hydrocarbons in urban rural fringe areas may be due to use in the fields or for termite control the total OCP were minimum <0.065 mg/kg and maximum of 0.26 mg/kg. Some chemicals may enter the Ami River directly from drains or from the atmosphere by volatization from the site of use. Other than DDT, aldrin, dieldrin, heptachlor, chlordane, endosulfan, HCB and BHC are present as contaminant. PCBs are Min 0.031 mg/kg and Max 0.055 mg/kg which was just above the detection limit and did not appeared to be a significant contaminant the source appeared to be the insulators etc, used for telecommunication. All the heavy metals were in some place or other above detection limits. On all sampling occasions results were highly variable. Hg was considerably high in the regions where heavy industry was found to be in greater number. Zn concentration was extremely high in some samples as 2300 mg/kg.

The fauna samples body tissue was analyzed (Table 3). The hydrocarbons concentration was high in two fishes *Gadusia* sp. and *Xenentodon* sp. Hydrocarbons enter the organism either by absorption across the surface of the body, particularly the respiratory tract and other absorptive surfaces or by ingestion of foods and particulates and subsequent absorption in the gut. The former has been considered to be the prime pathway of uptake of petroleum hydrocarbons in fauna (Bagg et al. 1981).

In fishes comparison of flow rate over the gill with drinking rates suggest that respiratory transfer is quantitatively more important of the two residues of the OCP and PCBs in fishes (Mah et al. 1989, Rogers et al., 1989; Whitehead et al. 1992). Some of the OCP and PCBs are shown in tissue of fauna in Table 4. Because the concentration of OCP found in the organs and tissues are primarily dependent on lipid contents and the liver has high concentration of oil thus the concentration of OCP liver can be two or three orders of magnitude higher than in muscle tissue in them. Transfer from water to all aquatic organisms can occur directly via respiration or by food chain transfer, in some cases with an accumulation from one trophic level to another (Blockwell et al. 1998).

Metals are natural components of Ami River flora and fauna. Many metals are essential for life but excessive exposure to many of these essential and non-essential metals may lead to adverse effect (Wiederholm and Dave 1989, SEPA (2000). The discussion will be centered mainly around heavy metals shown in the Table 5. Metals are present in river in complicated array of forms and each may generate a different short-term toxicity and has a characteristic availability. The pathway of metals in river water animals depends upon the chemical properties of the metal and physiology and biochemistry of the fauna. For instance in bivalve fresh water mussel chromium is absorbed readily from added sodium chromate not from chromic chloride. The table 5 indicates that Hg deposition is more evident in *Gadusia* sp. and *Unio* sp. 27 and 22 mg/kg of wet weight respectively. For many organisms the uptake rate of mercury is different in different forms of Hg, the organic Hg compounds are absorbed much more rapidly. The uptake rate is also modified by environmental factors such as sunlight, temperature, salinity, oxygen, saturation, carbon dioxide content, pH and the presence of dissolved organic matter, U.S. EPA (1997, 1999). For fresh water mussel metals may be preconcentrated on mucus and thus passed into the digestive system.

Conclusions

There are industrial sources as well as farming fields that discharge waste water effluents and are source of pollution in Ami River, which is situated in U.P.India at GIDA. Samples from the Ami River were collected from ten different sites and analyzed for hydrocarbons PAH's; BTEX; petroleum hydrocarbons, OCP and heavy metals. Most sample concentration were either beneath the detection limit or too low to be associated with biological impacts. The fauna samples of clam and some fishes body tissue was also analyzed. On all sampling occasions results were highly variable. Hg was considerably high in the regions where heavy industry was found to be in greater. Because the concentration of OCP found in the organs and tissues are primarily dependent on lipid contents and the liver has high concentration of oil thus the concentration of OCP in liver can be two or three orders of magnitude higher than in muscle tissue. Transfer of contaminant from water to all aquatic organisms can occur directly via respiration or by food chain transfer. Due to these effluents the disease, abnormalities including neoplasm and sudden kills of river fauna are most frequently recorded. The obvious solution to contamination is prevention, but inevitably even in the best-regulated systems, losses to the environment will occur, and there will be continuing pressure to dispose off some waste by dumping.

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Soil and sediment toxicity evaluation:current and prospective practice and applications / Évaluation toxicologique des sols et des sédiments: Pratiques courantes et futures et applications

Session Co-chairs/Présidents: G. Sunahara and/et G.L. Stephenson

Priorities for research and standardization of toxicology methods for assessing contaminant mixtures in Canadian soils. R.P. Scroggins[1], L. Novak[2], K. Becker-van Slooten[3], G.L. Stephenson[2], S. Siciliano[4], R. Kuperman[5] and D.A. Bright[6]. [1]Environment Canada, Methods Development and Applications Section, Ottawa, ON; [2]Stantec Consulting, Guelph, ON; [3]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [4]University of Saskatchewan, SK; [5]U.S. Army; and [6]UMA Engineering, Victoria, BC.

In February 2003, Environment Canada convened a 3 day workshop on the toxicological assessment of Canadian soils and development of standardized testing tools. The objective of the workshop was to identify priorities for method development, validation and standardization that would lead to a second generation of soil toxicology test methods for assessing the effects of contaminant mixtures in natural soils. Thirty-eight individuals (4 from Europe, 4 from U.S., 30 from Canada) participated in the workshop. The expertise assembled covered the disciplines of soil toxicology, microbiology, soil chemistry, earth sciences, soil ecology, plant physiology and the chemistry of contaminants in soil. The structure of the workshop incorporated a combination of plenaries and breakout working groups. There were three working groups: (i) Alternative Species and Procedure Modifications of Existing Terrestrial Toxicity Test Methods, (ii) Laboratory Functional Assays and Exposure Systems for Site Soil Assessment, and (iii) Physical or Biological Factors Influencing the Results of Soil Toxicity Tests. To facilitate the discussions, a pre-workshop questionnaire was developed for each work group. Questionnaire responses were received on 80+ issues, with over 130 pages of comments. The responses from all questionnaires were compiled and summarized into discussion documents, which were used to guide and focus the workshop discussions. The presentation will provide an overview of the workshop, including a summary of key priorities and recommendations that will lead to a new soil toxicity method development program.

Must individuals used in soil invertebrate toxicity tests originate from synchronous cultures? J.T. Crumb[1], G.L. Stephenson[1], N.C. Feisthauer[1], J.A. Miller[2], D. McLeay[3] and R.P. Scroggins[4]. [1]Stantec Consulting Ltd., Guelph, ON; [2]Miller Environmental Sciences Inc., Innisfil, ON; [3]McLeay Environmental Ltd., Victoria, BC; and [4]Environment Canada, Methods Development and Applications Section, Ottawa, ON.

The current draft Environment Canada biological test methods for soil invertebrates do not require that the individuals used in a test originate from synchronous cultures. Other agencies (ISO, OECD) recommend the use of test organisms from synchronous cultures. Comparative toxicity tests were conducted with contaminated site soils using individuals of *Eisenia andrei* (compost worm) and *Onychiurus folsomi* (springtail) from both synchronous and asynchronous cultures to determine whether synchronization of cultures was a prerequisite for testing. We predicted that synchronization would reduce the variability within treatment replicates of the reproduction metrics and result in a test being more sensitive (i.e., the probability of detecting an adverse effect would be greater). For reproduction tests conducted with *O. folsomi*, the results indicated that although the use of individuals from synchronous cultures produced a greater number of young the variability among replicates was slightly less when individuals from asynchronous cultures were used. Adult survival and fecundity were not significantly different between individuals from asynchronous and synchronous cultures, and the within treatment variability was comparable. Acute and chronic tests were conducted with *E.*

andrei from synchronous and asynchronous cultures using boric acid as a reference toxicant. The 7 and 14 d results of acute tests conducted with *E. andrei* indicated that there was no difference in sensitivity to boric acid based on culturing methods. The 14 d LC50 values were 3525.5 (3120.7–3982.8) and 3397.3 (3025.5-3814.8) mg H3BO3/kg soil d.w. for asynchronous and synchronous cultures, respectively. Preliminary data from the 63 d reproduction tests indicated little difference in 35 d survival between adult earthworms derived from synchronous and asynchronous cultures.

Comparison of experimental designs of earthworm *Eisenia andrei* **reproduction tests recommended by Environment Canada and the OECD**. K.D. Schneider[1], J.T. Crumb[1], G.L. Stephenson[1], P. Sibley[2] and R.P. Scroggins[3]. [1]Stantec Consulting Ltd., Guelph, ON; [2]Centre for Toxicology, University of Guelph, Guelph, ON; and [3]Environment Canada, Methods Development and Applications Section, Ottawa, ON.

Environment Canada's Method Development and Applications Section has developed draft test methods for toxicity assessment of contaminated soils to earthworms. In response to comments from a Scientific Advisory Committee, additional testing has been deemed necessary in order to address the source of variability reported in chronic earthworm data. European testing methodologies (OECD, ISO) have been able to meet validity criteria (e.g., <30% CV) lower than those observed with Environment Canada methods. Comparative earthworm reproduction tests were conducted concurrently, with one test following the Environment Canada draft biological test methods (December 2002), and the other following the experimental design described in the OECD Guideline for the Testing of Chemicals (OECD, 2000), in an effort to determine the effect that the nature of experimental design has on the variability of the test data. Both tests were conducted in artificial soil, using boric acid as the reference toxicant, and individuals of *Eisenia andrei* (compost worm) from asynchronous and synchronous cultures. We hypothesized that there would be no significant difference in the variability observed within treatment replicates of the reproduction metrics due to differences in the nature of the experimental design between Environment Canada and OECD test methodologies. An overview of the experiment will be presented and results for the asynchronous tests will be discussed, along with their implications for future development of Environment Canada's earthworm reproduction test.

Toxicological comparison of *Eisenia andrei* **in varying soil test systems recommended by Environment Canada.** J.I. Princz and R.P. Scroggins. Environment Canada, Methods Development and Applications Section, Ottawa, ON.

In 1995, Environment Canada embarked on a multi-year program to develop, validate, and publish national biological test methods specific to contaminated soils. Test methods were designed using species representative of terrestrial invertebrates (e.g., earthworms and arthropods) and plants inhabiting soil ecosystems in Canada. The validation of these methods is in the final stages. Environment Canada is continuing to assess these new soil test methods through reference toxicant testing using the recommended reference toxicant, boric acid. As part of the validation process for earthworm toxicity tests, acute lethality, acute avoidance, and reproduction testing were conducted. Acute avoidance studies were performed to examine differences in sensitivity and avoidance response of *Eisenia andrei* when exposed to contaminated soils using the recommended six-chamber avoidance test unit, and the alternative two-chamber avoidance test unit. The data from all tests were compared and assessed for validation of the proposed test methods. The use of both acute avoidance test units were compared and the performance of the different test chamber types examined.

Re-evaluation of the ecotoxicity values for CCME petroleum fraction 3: should fraction 3 be split? J.H. McCann[1], G.L. Stephenson[2], J.L. Roy[3] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Stantec Consulting Ltd., Guelph, ON; and [3]Imperial Oil Resources, Calgary, AB.

In Canada, the Canadian Council of Ministers of the Environment (CCME) developed Tier 1 standards for petroleum hydrocarbons in soils for four oil fractions based on n-alkane boiling point ranges. One fraction, (Fraction 3 - F3) however, often remains above the standard for the soil contact exposure pathway following remediation despite an apparent lack of toxicity in the field. Fraction 3 spans a large n-alkane boiling point range (>nC16-nC34), and it is hypothesized that the ecotoxicity can be attributed to the lower boiling point constituents (nC16-nC23) and F3b (>nC23-nC34). A black Chernozem soil was spiked with the individual fractions and both the acute and chronic toxicity of each fraction to three soil organisms were determined. The test species included one plant (northern wheatgrass, *Elymus lanceolatus*), one arthropod (Collembolan: *Onychiurus folsomi*), and one earthworm (*Eisenia andrei*). Endpoints assessed in the plants included emergence, and shoot and root length and biomass. Mortality and reproduction were assessed for the earthworm and arthropod species. The results will be discussed, as well as their implications for regulatory values and remediation.

Acute and chronic toxicity of the new explosive CL-20 in the earthworm *(Eisenia andrei)*. P.Y. Robidoux, G.I. Sunahara, K. Savard, Y. Berthelot, S. Dodard, M. Sarrazin and J. Hawari. National Research Council Canada, Biotechnology Research Institute, Montreal, QC.

Energetic materials such as 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) are polynitro-organic contaminants and are toxic to a number of ecological receptors including earthworms. In the present study, the lethal and sub-lethal toxicity of hexanitrohexaazaisowurtzitane (HNIW) also known as CL-20, in the earthworm (*Eisenia andrei*) were evaluated. Two sandy-type natural soils, a forest soil (RacFor2002, 20% organic carbon, pH 7.2) and the Sassafras sandy loam (SSL, 0.33% organic carbon, pH 5.1) soil were used in this study. Results showed that CL-20 was lethal at concentrations ≥90.7 mg/kg in the SSL soil, but not lethal at concentrations ≤125 mg/kg in the forest soil. Effects on the reproduction parameters, such as a decrease in the number of juveniles after 56 d of exposure, were observed at ≥0.2 mg CL-20/kg dry SSL soil (nominal concentration). Higher concentrations (≥1.6 mg CL-20/kg dry soil) were needed for the same effect in the forest soil. Other sublethal effects in the earthworms, such as the lysosomal membrane fragility (measured using the neutral red assay), were also observed. Data indicate that toxicity can be decreased in soils favoring CL-20 adsorption (high organic carbon content).

Compared to TNT, RDX and HMX, CL-20 appears to be the most toxic explosive compound studied using the earthworm *E. andrei*.

Toxic effects of polynitro-organic compounds on enchytraeids (Oligochaeta) in freshly amended natural soils. S. Dodard[1], J. Hawari[1], M. Sarrazin[1], P. Gong[1], L. Paquet[1], G. Ampleman[2], S. Thiboutot[2] and G.I. Sunahara[1]. [1]National Research Council Canada, Biotechnology Research Institute, Montreal, QC; and [2]Defense Research and Development Canada.

Sites contaminated with military-unique and highly energetic compounds such as TNT (2,4,6-trinitrotoluene), RDX (1,3,5-trinitro-1,3,5-triazacyclohexane) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) pose a challenge to ecotoxicology risk assessors and bioremediation specialists. Very little is known about the sublethal effects of these polynitro organic compounds in soil organisms. A new heterocyclic polynitramine propellant and explosive called hexanitrohexaazaisowurtzitane (HNIW) or CL-20 has been recently developed, but its toxicity in earthworms and enchytraeids has not been well characterized. The effects of CL-20 on the survival and reproduction of enchytraeid species (*Enchytraeus albidus* and *Enchytraeus crypticus*) were examined and compared to other explosives such as TNT, RDX and HMX. These studies were also carried out using different exposure matrices including a freshly amended Sassafras sandy loam soil, an agricultural soil, and a composite agricultural-forest soil. Our data indicates that CL-20 is more toxic to enchytraeids than TNT, RDX and HMX, and should be considered as a potential reproductive toxicant to soil invertebrates.

Duckweed, collembols and more - application of effective population growth tests in soil ecotoxicity testing. M.F. Eberius. LemnaTech GmbH., Wuerselen, Germany.
While aquatic bio-testing is very common and has a long successful history testing of soil and solid media is a relatively new field. Using relatively new standardised bio-tests like duckweed and collembols combined with specifically developed sample pre-treatment and population measurement methods gives the option for an effective application of population growth tests. Use of soil water suspensions and loading series adds an important tool to apply standardised duckweed testing from widespread first screening of samples to detailed risk assessment on biologically available toxic potential of a soil. Image processing as tool to gain the results in combination with statistically sound calculation of inhibition (growth rates) gives high reliability and a maximum of specific knowledge on mode of action of the contaminants. Results showed, that there was no general difference between soil and soil elutriate testing with plants. Use of image processing for counting high numbers of soil dwelling organisms like collembols makes these test quite effective and offers a lot of information beyond mere numbers as size distribution gives additional sublethal information. This concept of whole population testing without need to restrict numbers of offspring by test design is especially helpful to cope with soil inhomogenicity and growth variabilities. Further test systems to apply this concept in near future are daphnia, nematodes and enchytraeids.

Kinetics and mechanisms of PAHs sequestration in marine and freshwater sediments. D. Brion et É. Pelletier. Institut des Sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, Qc.

Abstract

Polycyclic aromatic hydrocarbons (PAH) are known to be persistent in sediments and are expected to sequester by aging process responsible for the change in the availability of PAHs. For sequestration rate study of PAH, natural sediment slurries were spiked with [^{2}H]-PAH and re-extracted periodically with a high molecular weight surfactant solution (Brij® 700) representing the contaminants available fraction. Dissolved [2H]-PAHs were first adsorbed on particles surface within 4-7 d following their molecular weight or hydrophobicity character. Adsorbed molecules became slowly sequestered into dense organic matter or inaccessible microsites and gradually were more difficult to extract. An empirical model based on a three-compartment dynamic system in which PAHs are taken up from water and gradually sequestered by particulate matter, was developed to quantify the sequestration rate constants of toxicants. In first approximation, the three-compartment system was simplified to a first-order consecutive-irreversible two-stage reaction. Adsorption rate constants were between 0.056 -0.017/h and were approximately ten times faster than sequestration rate constants (0.02 -0.001/h). Light PAHs were the fastest to enter in the sequestration process. The presence of a large quantity already sequestered PAHs seem to play a determining role in the sequestration of new coming PAHs.

Introduction

Polycyclic aromatic hydrocarbons PAHs is known to be generated by the incomplete combustion of organic matter in natural and anthropic processes such as forest fires, internal combustion engines, and industrial activities. PAHs are also known to be toxic and persistent in marine and freshwater sediments. PAHs as well as other persistent organic compounds demonstrated a declining availability to aquatic organisms with increased residence time in sediments (Hatzinger and Alexander 1995). This aging process, often called sequestration, is responsible for changes in their availability and can be attributed to a slow migration of PAH molecules into condensed organic matter and inaccessible microsites (Steinberg et al. 1987). When sequestered, molecules are inaccessible to organisms and to extracellular microbial enzymes, diffusion out of these sites is extremely slow and their availability is mainly governed by the slow rate of molecular desorption. During this period, only very low concentrations of contaminants will be available for organisms (Man and Alexander 1998).

Although a large number of studies have been reported on the adsorption/desorption process of various PAHs on a variety of lab-made particles and natural sediment or soil (Karickhoff 1980, Nam and Alexander 1998, Valsaraj and Thidodeaux 1999). not much have been reported on sequestration kinetics. The fate of hydrophobic organic compounds in natural water is dependent on their sorptive behaviour, and PAHs are thus expected to remain sorbed to the sediment due to their non-ionic and hydrophobic properties (Achuler and Lydy 2001). Little is known about the kinetic of molecular diffusion into sediment matrix and also on the first steps of the sequestration process.

Extensive Soxhlet extractions using dichloromethane (DCM) and acetone are currently in use to quantify PAHs in contaminated sites (Guerin 1999). These extraction techniques measure total PAH concentrations and are not representative of the real exposure of benthic organisms to toxicants. Non-exhaustive extraction techniques were developed for a better evaluation of the available fraction of PAHs (White et al., 1997, Reid et al. 2000). However, these techniques did not take into consideration the fact that microorganisms produce surfactants or other emulsifiers which can partially dissolve particulate organic matter and thus permit a cellular entrance of small and large organic molecules (Park et al. 2002). It is known that surfactants, above their critical micelle concentration, can solubilize non-ionic organic compounds added to soil-water mixtures (Liu et al. 1991). Accordingly, a new technique was developed recently (Barthe 2002) using a high molecular weight surfactant (Brij® 700) to desorb the labile fraction of hydrophobic molecules from sediment matrix and estimate its availability.

The purpose of this study was to determine the kinetic of sequestration of a number of PAHs and also to investigate factors such as grain size, carbon content, contamination level, hydrophobicity or molecular structure that may influence sorption kinetics. The surfactant (Brij® 700) extraction technique determined extractible [^{2}H]-PAHs concentration as a function of time and represented mainly the available PAH fraction sorbed on sediment. A kinetic model on the sequestration phenomenon based on a three-compartment model was developed to evaluate the kinetic constants. In some sediment, the intensity of sorption is known to be dependent on the origin, molecular weight, polarity and molecular configuration of the humic substances (Chiou et al. 1986). For different soils, carbon-normalized binding constants (K_{oc}) of a single hydrophobic organic compound may range over an order of magnitude, and such variations has been attributed to compositional differences in the organic matter, such as polarity and aromaticity (Murphy et al. 1990). Thus, many different factors can affect sequestration kinetics such as the amount of organic carbon or clay content present in soil or sediment (White et al. 1997). Organic carbon content in soil has been identified as a major determinant factor for aging (Nam et al. 1998). In experiments, parameters such as temperature, light, microbial activity and the PAH/sediment ratio were controlled in order to reduce their influence in the kinetic process.

Materials and Methods

A three-compartment model: Sequestration can be described by two major molecular processes where adsorption is the first step and absorption or sorption is the final stage. A three-compartmental thermodynamic system shown by Fig. 1 can describe these successive phenomenons. The adsorption process is the main precursor to sequestration in which hydrophobicity drives dissolved molecules through a solution onto particulate organic matter. Then, adsorbed molecules will migrate slowly into condensed organic matter defining sorption or sequestration (Steinberg et al. 1987). In this model, the first compartment *W* is defined as the amount of dissolved PAHs which can be adsorbed to particles in the second compartment *Ads* and finally sequestered in the compartment *Seq*. We hypothesized that surfactant extracted only PAHs weakly bound onto particle sediment which represented the available not sequestered fraction. It was also assumed that adsorption was an irreversible reaction because of the high hydrophobicity of aromatic hydrocarbons. For a sequestered molecule, the kinetic of desorption should be a slow process governed by molecular diffusion and redissolution in the aqueous phase. As a result, the slow rate of desorption from sequestered PAHs can be neglected in short term experiment (few weeks) and then sequestration can be considered as an irreversible reaction. With these conditions in place, the system was then defined as a first-order consecutive-irreversible two-stage reaction. Fig. 1 describes the transport of PAHs in this kinetic model. Symbols *W*, *Ads* and *Seq* stand for the amount of PAHs in their respective compartment linked by two rate constants k_1 and k_2. This mechanism can be described by the following set of equations:

$$-\frac{d[W]}{dt} = k_1[W] \tag{1}$$

$$-\frac{d[Ads]}{dt} = k_1[W] - k_2[Ads] \tag{2}$$

$$\frac{d[Seq]}{dt} = k_2[Ads] \tag{3}$$

At time $t = 0$, only dissolved PAHs were present in this system then $[W]_0$ is defined as the initial quantity of the reacting material *W*. Equation 1, which represents the rate of disappearance of *W*, gives after integration

$$[W] = [W]_0 e^{-k_1 t} \tag{4}$$

The concentration of *Ads* is computed from Equation 2, which is a first order linear differential equation. Written in the more familiar form, Equation 2 becomes:

$$\frac{d[Ads]}{dt} + k_2[Ads] = k_1[W] = k_1[W]_0 e^{-k_1 t} \tag{5}$$

when solving this equation we obtain

$$[Ads] = \left[\frac{k_1[W]_0}{(k_2 - k_1)}\right]\left[e^{-k_1 t} - e^{-k_2 t}\right] \tag{6}$$

At any time t, $[W] + [Ads] + [Seq] = [W]_0$, then Equation 3 will yield

$$[Seq] = \left(1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1}\right)[W]_0 \tag{7}$$

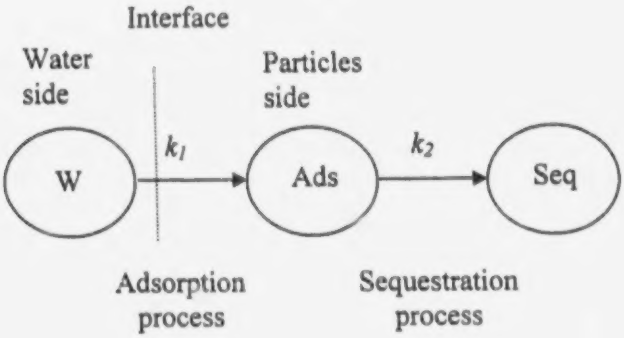
Interface
Water side
Particles side
W
k_1
Ads
k_2
Seq
Adsorption process
Sequestration process

Fig. 1. Schematic diagram of the three-compartment model of sequestration defining kinetic parameters.

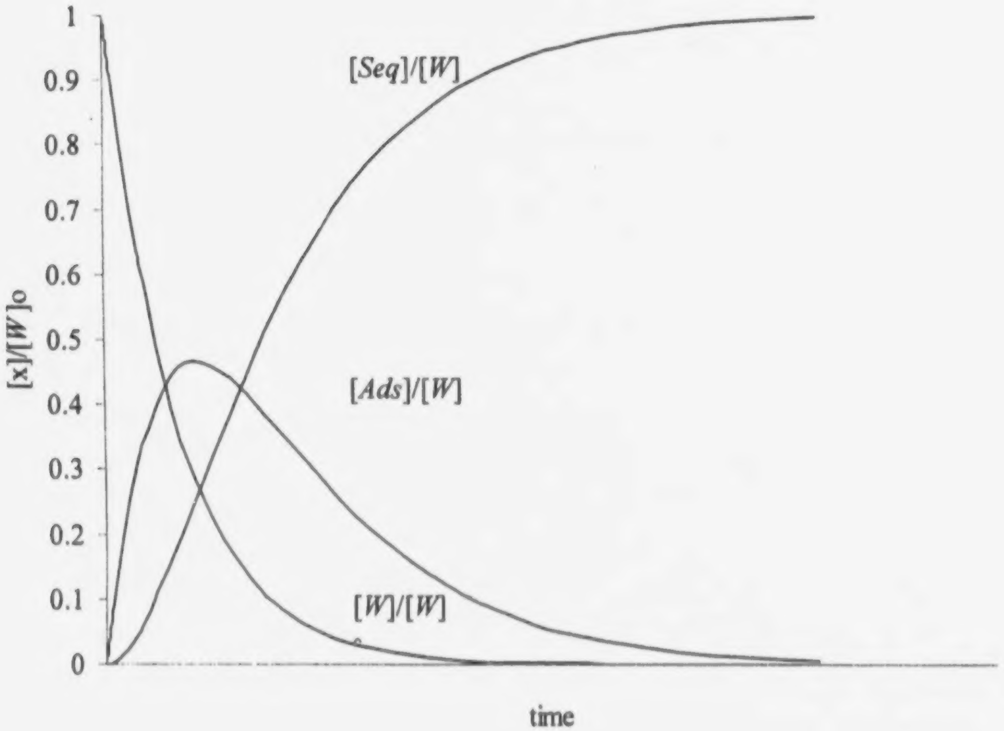

Fig. 2. General kinetic behaviour of the three compartments driven by a first-order consecutive-irreversible two-stage reaction model.

Fig. 2 shows the kinetic behaviour of the three compartments from the first-order consecutive-irreversible two-stage reaction model. In first approximation, experimental data on the amount of extractible [^{2}H]-PAHs behaved like the *Ads* compartment wherein the increasing concentration was fast and followed by a slow decrease. After some trials, the best fitting curve for experimental data was obtained and equations similar to *Ads* kinetic equation were suggested. SPSSTableCurve software provided a non-linear regression with an equation in which parameters can be directly related to the parameters from the kinetic model. Considering the kinetic of molecular diffusion resulting from the hydrophobicity driving force, the rate k_1 might be superior to k_2 because the intra particular molecular diffusion is a much more slower process. The rate constants were determined by a non-linear regression with SPSSTableCurve 2D v5.0 software.

Surfactant extraction: The method was developed by Barthe 2002. One gram of wet sediment and 10 ml of Brij® 700 solution (5.25 mM) were added in a 50 ml Teflon tube precleaned with DCM. After overnight shaking, the slurry was centrifuged for 20 min at 3000 rpm. The aqueous phase was then extracted with 20 ml (2x10 ml) of dichloromethane and the extracts combined into a glass tube. The sample was evaporated to 0.5 ml under a nitrogen stream in an ice-bath. Pentane (2 ml) was then added to the sample and the volume was reduced to 1 ml. The sample was transferred to the top of an Enviropack® 18 (3 ml/500 mg) column and eluted with 5 ml of a 90:10 (v/v) pentane:DCM mixture. The eluate was then evaporated under nitrogen stream and in an ice-bath to 0.5 ml, 2 ml of acetonitrile was added, and the evaporation resumed until the volume reached 1 ml Sample was then analysed by HPLC-Fluorescence. All analyses were done at a constant flow rate of 0.8ml/min with a mixture of nanopure water and acetonitrile. The pump program began at 75% of acetonitrile and raised to 95% at 10 min then raised to 100% at 20 min and held it for 10 min before ending to 75% acetonitrile after a total run of 35 min. For the fluorescence detector, the excitation wavelength was settled at 280 nm and the emission wavelength was 340 nm during the first 5.50 min for lighter PAHs and 410 nm until the end of program (25 min) for heavier PAHs. The marine sediment core was sampled using a multi-core from the Saguenay fjord (Quebec, Canada). The core was sub-sampled into 4 cm sections. Freshwater surface sediment was collected from the St-Louis River (Quebec, Canada).

Sequestration experiments: Ten grams of wet sediment and 50 ml of deionised water were added into a beaker. The slurry was sterilised with a germicidal lamp UV-C (254 nm) tube) at 4°C for 48 h. One ml of a 10 μg/ml solution of 16 EPA [^{2}H]-PAHs cocktail in benzene was evaporated under nitrogen to 0.1 ml in an ice-bath, and 0.4 ml of acetone was added for a better dissolution of labelled PAHs in the aqueous phase. Sediment slurry was then spiked with this 0.5 ml solution of the 16 EPA [^{2}H]-PAHs cocktail and another 50 ml of deionised water was added giving a final [^{2}H]-PAH concentration of 100 μg/L. Beakers were then hermetically closed and slurries were stirred at 4°C in the dark for the time of the experiment. Sampling volume of sediment slurry spiked with labelled PAHs was 10 ml performed with a sterile pipet in order to keep the same ratio sediment/water in the beaker. Adsorption by the glass was neglected because of the surface presented which is approximately 1% of particles surface calculated with $r=1x10^{-4}$ cm as a mean of the radius of one particle, a mean of the density of the dry sediment of 3 g.cm^{-3} and with a 40% of humidity for wet sediment.

Results

Sediment and PAHs characteristics: Total PAHs (16 EPA priority PAHs) concentrations in the sediment as determined by a DCM extraction and by the GC-MS analysis are reported in Table 1. It is clear that RIV was more contaminated by PAHs with a concentration of 4191.5 ng/g compared to SAG (383.9 ng/g). RIV was sampled at few meters from an industrial effluent and SAG was sampled in the middle of a bay at about 5 km from an industrial complex. The PAHs proportion between these two sediments was slightly different with less fluorene and chrysene and with a higher proportion of phenanthrene, fluorene and pyrene in SAG compare to the highly polluted site of RIV. As such, the presence of high concentrations of PAHs in sediment increases the proportion of organic

Table 1. Grain size distribution, carbon content and concentration (data from GC-MS ± 0.5 ng/g d.wt.) of the 16 PAH classed by EPA in two sediments.

	RIV	SAG
% Silt	85.3	77.4
% Clay	14.7	12.6
% C	7.10	1.89
C/N	184.4	18.9
Naphtalene	8.2	7.4
Acenaphtylene	6.0	N/A*
Acenaphtene	140.4	27.4
Fluorene	133.1	6.2
Phenanthrene	168.8	68.5
Anthracene	20.5	20.2
% Sand	0	10
Fluoranthene	173.9	65.1
Pyrene	132.5	53.8
Benzo(a)anthracene	214.6	13.9
Chrysene	1952.1	16.6
Benzo(b,k)fluoranthene	385.3	19.0
Benzo(a)pyrene	839.9	39.8
Benzo(ghi)perylene	8.6	20.8
Dibenzo(ah)anthracene	6.6	4.4
Indeno(1,2,3-cd)pyrene	1.1	20.8
Total PAH	4191.5	383.9

*Below detection limit

matter and might affect sequestration process.

The high content of carbon (7.10%) (Table 1) in RIV with total PAHs concentration of 4191.5 ng/g may explain the high ratio of C/N. The C/N value of 184.4 in RIV indicated a very low content of nitrogen which generally means the presence of an old organic content with low energetic value usually related to a polluted site. Sediment characteristics showed us that SAG samples had much lower carbon content (1.89%) than RIV with a total of PAHs of 383.9 ng/g (Table 1). Considering the standard error (±2%) in grain size values sediment SAG and RIV was not significantly different in mineral composition (clay, sand and silt).

The two classes of polycyclic aromatic hydrocarbons in this study are hydrophobic, and the molecular surface related to the molecular weight and the molecular volume is highly correlated to the log Kow which represents their hydrophobicity. Fluorene with a log Kow value of 4.18 and a surface of 194 Å^2 (Table 2) is the less hydrophobic PAH in the fluorene-like group. Phenanthrene is also the less hydrophobic molecule with a log K_{ow} value of 4.52 in the phenanthrene-like group. All molecules are planar because of the atomic orbital hybridation except fluorene which has one carbon in *sp*^{3} hybridation.

Experiments: The observation of raw data from each experiment rapidly showed that changes in the amount of [^{2}H]-PAH extracted was a function of time provided a distribution with a maximum value similar to the curve [*Ads*]/[*W*]$_0$ showed in Fig. 2.

Table 2. Physical properties of both PAH structural families.

PAH	Mol. Weight g/mol	Sol. mg/L	Log K_{ow}	Log K_{oc}	Mol. Surf. (Å^2)	Mol. Vol. (Å^3)
Phenanthrene	188.2	0.4-1.6	4.52	3.97	199.3	170.6
Pyrene	212.3	0.16	5.18	4.52	213.5	186
Benzo(a)pyrene	264.3	0.003	6.31	5.48	255.6	228.6
Benzo(ghi)peryle.	288.3	0.00026	7.23	6.26	266.9	244.3
Fluorene	176.2	1.9	4.18	5.47	194	160.4
Fluoranthene	212.3	0.1-0.3	5.33	4.65	218.6	187.7
Benzo(k)fluorant.	264.3	0.0008	6.84	5.92	264.9	231.1

Each kinetic data series result from a compilation of 2-4 differents experiments with the same sediment and under the same conditions. SAG was a compilation of three 28 d experiments and one 7 d experiment. Data of RIV were composed of two 7 d experiments and one 28 d experiment. Both SAG and RIV samples have their maximum [^{2}H]-PAH concentration extracted by Brij® 700 between 0.12-0.29 μg/g (wet weight) before the 100 h experiment (Table 3). In RIV, both PAHs structural families show the same general trend which was characterized by a decrease of the maximum extractible concentration of [^{2}H]-PAH with the increase of PAH molecular size. The time to reach the maximum was also increasing with the size of PAH. SAG data have same trends but the maximum concentrations were reached between 72 and 96 h which are longer than RIV. SAG

Table 3. Time to reach (h) the mean maximum concentration of extractible PAH (µg/g wet wt.).

	RIV		SAG	
	Time	Max (±0.03)	Time	Max (±0.03)
Phenanthrene	48	0.29	72	0.29
Pyrene	72	0.20	96	0.25
Benzo(a)pyrene	72	0.14	96	0.26
Benzo(ghi)perylene	72	0.14	96	0.23
Fluorene	48	0.24	96	0.12
Fluoranthene	72	0.18	96	0.25
Benzo(k)fluoranthene	72	0.15	96	0.29

indicated that the maximum concentration of extractible PAH decreased with the increasing of PAH size in phenanthrene-like structures but in fluorene-like structures the opposite was observed. These results suggested that the two sediments affected differently the sorption of [^{2}H]-PAHs.

Fig. 3 shows the general trend of *Ads* compartment and the non linear regression applied to experimental data with two different [^{2}H]-PAH. Using software, it became possible to determine the kinetic parameters for each studied PAH and each sediment. These results are summarised in Table 4 and show how $[W]_0$, k_1, and k_2 changed with the structure and size of the PAH molecule and nature of the sediment tested. With freshwater contaminated sediment RIV, smaller molecules such as phenanthrene, pyrene, fluorene and fluoranthene have higher $[W]_0$ and k^1 than bigger molecules such as benzo(ghi)perylene and benzo(k)fluorenthene. Rate constant k_2 defining the rate of sequestration is usually one order of magnitude smaller than k_1 standing for the rate of adsorption on to particles from the water compartment *W*. The situation looks different for the low contaminated marine sediment (SAG) where the nature of the PAH molecules seems not to be a determining factor in the sequestration process. $[W]_0$ is not decreasing in the increasing size of the molecules as seen with RIV and even increased from fluorene to benzo(k)fluoranthene. Both k_1 and k_2 do not show any peculiar trend as a function of the shape and the size of the PAH molecules. Again the sequestration rate, k_2, is still roughly one order of magnitude lower than the adsorption rate. It should also be observed that correlation coefficient (R^2) of best-fitting curves are usually better in sediment SAG than RIV. That might be partly attributed to a reduced number of data available for RIV, but it seems also to reflect the presence of a more complex sedimentary environment where the presence of a high level of organic matter and indigenous PAHs seem to play a determining role on the kinetic parameters.

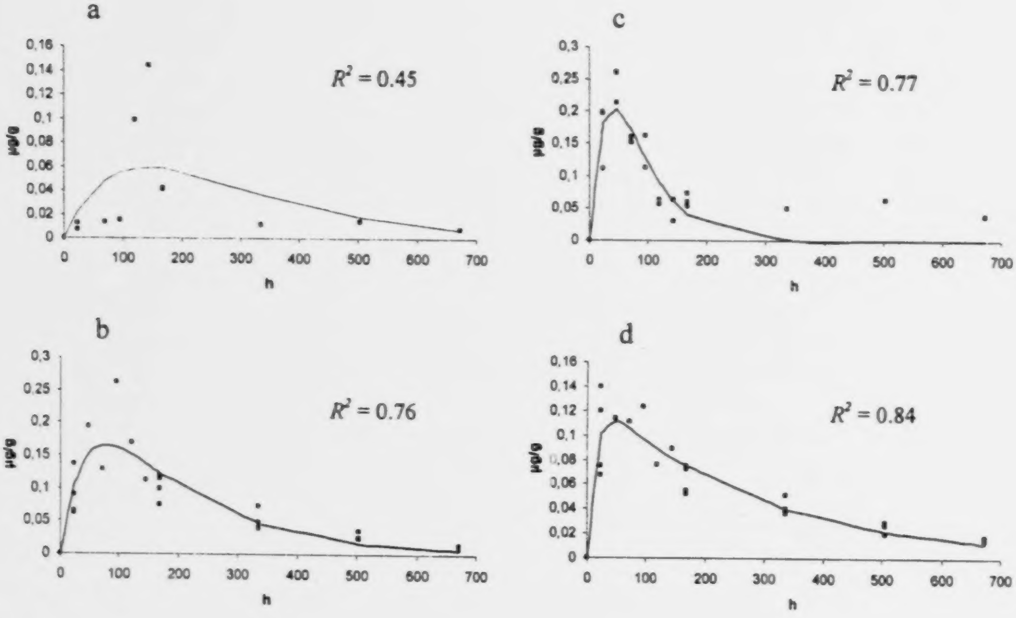

Fig. 3. Examples of non-linear regression results on experimental data using the Equation 6 for benzo(a)pyrene (BaP) (a) in RIV and (b) SAG, and fluorene (c) in RIV and (d) SAG. Parameters were reported in Table 4.

Table 4. Values (± SE) of kinetic parameters calculated from experimental data (SAG: 4 experiments combined; RIV: 3 experiments combined) by non-linear regression analysis for the three compartment model.

	RIV				SAG			
	$[W]_0$	k_1	k_2	R^2	$[W]_0$	k_1	k_2	R^2
Phe	0.277 ±0.070	0.056 ±0.039	0.006 ±0.003	0.45	0.267 ±0.031	0.037 ±0.011	0.003 ±0.001	0.76
Pyr	0.187 ±0.030	0.040 ±0.019	0.002 ±0.001	0.48	0.217 ±0.023	0.045 ±0.013	0.0025 ±0.0005	0.72
B*a*p	0.132 ±0.057	0.017 ±0.013	0.003 ±0.003	0.45	0.266 ±0.062	0.023 ±0.009	0.006 ±0.002	0.76
B(ghi)	0.122 ±0.034	0.023 ±0.015	0.002 ±0.002	0.42	0.173 ±0.024	0.033 ±0.011	0.0024 ±0.0006	0.62
Fluoren	0.474 ±0.565	0.029 ±0.040	0.020 ±0.026	0.77	0.131 ±0.013	0.070 ±0.023	0.0037 ±0.0007	0.84
Fluoran	0.193 ±0.047	0.029 ±0.015	0.004 ±0.002	0.54	0.221 ±0.022	0.039 ±0.010	0.0026 ±0.0005	0.77
B(k)flu	0.011 ±0.027	0.024 ±0.014	0.001 ±0.001	0.44	0.226 ±0.029	0.039 ±0.010	0.0027 ±0.0007	0.69

Phe:phenanthrene;Pyr:pyrene;B*a*p:benzo(a)pyrene;B(ghi):benzo(ghi)perylene;Fluoren:fluorene; Fluoran:fluoranthene; Bz(k)flu :benzo(k)fluoranthene. Units of rate constant : h^{-1} ; $[W]_0$ in µg/g wet sediment.

We know that at any time *t* of the three-compartmental kinetic model, $Ads+W+Seq=1$. Thus, when the extraction of adsorbed PAHs is made there is always a proportion of PAHs in the two other compartments which are not measured. Only 10-30% of the initial concentration of [^{2}H]-PAH was recovered by the extraction. The described model works if there is no PAH exchange with another compartment. In order to prevent a PAH loss in the system, every precautions were taken. We assumed that the loss due to glass adsorption was minimal (1% of total adsorption surface). Experiments were run in dark and at a constant temperature of 4°C in order to minimized possible PAH loss due to photodegradation and evaporation. During the experiment a small vortex was created in order to prevent the formation of a hydrocarbon micro-layer at the surface and ensure a good mixing of slurry. The solubility of benzo(a)pyrene, benzo(ghi)perylene and benzo(k)fluoranthene (Table 2) was lower than requested 100 µg/L. Acetone (3164 mg/L) was added for a better dissolution of PAHs. This high ratio of acetone toward PAHs molecules allowed dissolving more easily PAHs in the aqueous slurry. In addition, before spiking sediment with [^{2}H]-PAH, the system was placed under a UV-C light for 48 h in order to prevent PAH loss due to bacteria activity.

Discussion

As seen from Fig. 3, the first part of the curves revealed an increasing of [*Ads*]. This informs an increase of [^{2}H]-PAH presence onto sediment particles as dissolved labelled PAHs became more and more adsorbed to particles. Because of their high hydrophobic properties, dissolved [^{2}H]-PAH are considered to bind rapidly to particles. In RIV sample, the highest maximum concentration of [^{2}H]-PAH extracted was exhibited by phenanthrene with a mean value of 0.29 μg/g (w.wt.) reached in 48 h (Table 3). In fact, the maximum [^{2}H]-PAH extractible concentration decreased with the increasing molecular weight between dissolved and adsorbed PAHs compartments. In SAG, the maximum extractible mean concentrations of [^{2}H]-PAH were all reached between 72 and 96 h and the highest were exhibited by phenanthrene and by fluoranthene and benzo(k)fluoranthene. These results tell us that fluorene behaved differently from other PAHs with sediment SAG. Rate constants k^1 were decreasing as PAHs molecular weights increased in both sediments. Considering the results and their relative extractability, lighter [^{2}H]-PAHs were adsorbed more rapidly than heavier ones. Thermodynamically advantaged, small molecules will be rapidly transferred from the dissolved compartment to the adsorbed compartment by diffusion process as seen by high values of k_1. Conversely, they can easily be removed from surface particle by surfactant for the same thermodynamical reason. Generally, for both phenanthrene-like and fluorene-like structures, there was an apparent increasing adsorption rate order in the two samples which correlated to decreasing hydrophobic order.

Liquid/solid sorption is a mass transfer process between the bulk fluid phase and the adsorption sites in a porous sorbent. The sorption rate is governed by a mass transfer resistance caused by the fluid film surrounding the solid interface, and by internal macro and microporous diffusional resistances (Valsaraj and Thibodeaux 1999). This first step of water/solid transfer is rapid in comparison to the intra-particle diffusion process which is the last step of absorption also termed as sequestration. As the hydrophobicity of the compound increases, the particle-side resistance term becomes less important, and the water film is the controlling factor of the mass transfer rate (Valsaraj and Thibodeaux 1999). This phenomenon is consistent with the fact that heavier [^{2}H]-PAHs were less extracted by Brij® 700 and thus suggests a more efficient sorption. Our results corroborate previous results of Karichoff (1980), who studied PAHs. Compounds with higher hydrophobicity (i.e., higher logK_{ow}) had slower uptake rates into sediment. This latter finding is important, because if local sorption equilibrium between molecules dissolved in particle fluids and those sorbed locally in the aggregates is always established, the chemicals with higher partition coefficients are predicted to penetrate the natural sorbent aggregates more slowly if diffusive transport occurs primarily in the intra-particular fluids. The compounds with higher molecular weights will penetrate slowerly because of their lower diffusivity. Since molecular diffusivity is inversely proportional to one-third power of molar volume (Cussler 1997), differences in the solution diffusivities do not vary greatly among these compounds. Therefore, the effects of hydrophobicity dominate the variation of sorption rate for different compounds. The partition of organic carbon (K_{oc}) related to hydrophobicity (log K_{ow}) is highly correlated to the aromaticity of organic substances in sediment (Gauthier et al. 1987) and differed with the quality, chemical and sterical properties of the organic matter. It is clear that the quality of the sorbent has to be considered in hydrophobic organic adsorption processes.

The second part of the curves revealed a slow decrease of extractible [^{2}H]-PAH concentrations. While keeping constant all experimental conditions, [^{2}H]-PAH became more and more difficult to extract. The rate constant k_2 determined with the non-linear regression for each [^{2}H]-PAH (Table 4) are related to sequestration into the sediment matrix. Considering the standard error, the sequestration rate constant k_2 were similar between compounds in each sediment except fluorene in RIV. The mean value of k_2 was 0.003/h which is ten times lower than k_1. However, fluorene was rapidly sequestered in RIV with a high k_2 value of 0.02/h.

The calculated $[W]_0$ value decreased as the PAHs surface or volume increased except for fluorene-like structures in SAG wherein fluorene was the lowest. Considering the results, fluorene was two times less extracted by Brij® 700 in SAG than benzo(k)fluoranthene and fluoranthene. This molecule among all PAHs, has obviously a different kinetic behaviour. Fluorene has one carbon in sp^3 hybridation giving a non planar molecule. This sterical hindrance may affect adsorption into sediment matrix which is composed of molecules with a certain sterical configuration (Chiou et al. 1986). The delay in sorption process created by this sterical interference might be long enough to expose more fluorene to surfactant extraction increasing rate constants especially when the organic content is high. Conversely, when sequestered, this sterical hindrance might reduce the extraction efficiency in SAG and was probably caused by a best penetration in microsites. Therefore, the planarity of the molecule might be an important factor in sorption or molecular diffusion because of hindrance constraint affecting the sequestration (Chiou et al. 1986).

Experiments with sediment from SAG provided the best coefficient of determination ($R^2 \geq 0.62$) compare to RIV ($R^2 \geq 0.42$). The three compartment model does not consider the competition behaviour between native PAHs and labelled PAHs for the adsorption sites. The presence of other hydrophobic organic compounds (HOC) from aqueous solution of soils has been found to affect the uptake of individual HOCs and is considered as a competitive behaviour (McGinley et al. 1993). White (1999) has found in experiments with soil that the addition of pyrene increased the physical availability of phenanthrene by a competitive displacement of phenanthrene from sorption sites. RIV was more polluted and sites readily charged in PAHs. The presence of industrial carbon residues may affect greatly sorption comportment of hydrophobic contaminants. The competitive behaviour for adsorption sites was then greater than SAG, thus [^{2}H]-PAHs was less extracted within time. Therefore, during the short period of adsorption, [^{2}H]-PAHs in RIV was more available for extraction reducing the significant level (R^2) of the model. As such, the fitting of the model was better for the low polluted site SAG.

According to data, sequestration tends to an asymptotic value after 17 d of experiment but a little proportion of [^{2}H]-PAH (approximately 5%) was still extractible after 400 h. It was possibly caused by an existing equilibrium between sequestered and adsorbed PAHs. It is known that hydrophobic compounds may desorb but it is a very slow process compared to the sequestration. The experiment allowed us to reveal partially this equilibrium but stopped before it was established. Only a longer experiment and a model taking in account desorption may bring more insights on this phenomenon. Except for fluorene, the sequestration rate k_2 in this kinetic model was similar for all PAHs indicating that factors like the origin of the sediment, grain size, organic carbon content and the global chemical composition had little or no selective effects on compounds. Either the model was not appropriate for the second part of the kinetic curve or sequestration factors are more difficult to identify even to quantify. Perhaps the investigation should be more oriented at molecular level for sequestration process. A better and complete characterization of the organic matter will be necessary in order to identify new determinant factors.

A possible factor to be taken in account is the difference in aromatic properties between the two PAHs families studied. Adsorption is an association of hydrophobic organic compounds with particulate organic matter. Because of their weak solubility, the adsorption of PAH is a combination of two forces: Van Der Waals forces and a thermodynamic gradient determined by the hydrophobicity driving them out of the solution (Voice and Weber 1983). For neutral PAH molecules, adsorption is dominated by attractive forces of Van Der Waals between instantaneous and induced dipole moments of molecules (Gauthier et al. 1987). Dipole moment depends upon the polarizability and can be estimated by the sum of bond moments in the molecule. An increase in the number of C=C bonds should contribute the molecular polarizability and thereby increases the Van Der Waals attraction strength. As a result, heavier PAHs with more C=C bonds should adsorb efficiently to sediment particle and their extractability with Brij® 700 should be reduced compare to lighter PAHs. As such, larger PAHs can be strongly sorbed to soot and other particles and became less available to organisms (McGroddy and Farrington 1995, Neff and Burns 1996).

The extraction of individual PAH from sediment slurry by micellar surfactant solution involves three steps: matrix diffusion of PAH molecules out of the sediment particles, mass transfer at the interface water/sediment, and partitioning into the micellar phase. The last step is known to be extremely fast and does not exceed few microseconds but the first two steps are considered to be the rate-limiting steps in term of hours (Geheln and De Schryver 1993, Fu et al. 1994). Matrix diffusion appeared to be the controlling factor in the solubilization of PAHs (Yeom et al 1996). Thus, light individual PAH molecules are much smaller than surfactant micelles and it suggests an increase of the solubilization and subsequently a faster transport characterized by a larger k_1, related also by a better diffusion from sediment matrix in comparison to heavier PAHs.

Another possible factor playing a role in natural sediment is the nanoporosity and their relative hydrophobicity which may affect sequestration kinetics (Alexander 2000). Pores with a diameter inferior to 1000 Å are present in every sediment (Chung and Alexander 1999) and the studied [^{2}H]-PAHs had a volume in between 170 Å3 and 244 Å3 giving respectively, with an approximate diameter of 7 Å and 8 Å (Table 2). This pore size particularly fit well with these organic molecules. Tests with beads with a pore diameter in between 25 and 150 Å demonstrated that adsorbed phenanthrene desorbed rapidly from these pores if the pore surface was non-hydrophobic (Nam and Alexander 1998). This is consistent with the fact that highly hydrophobic material such as PAHs are sorbed to organic surface and their concentration is highly correlated to organic content.

Conclusions

The aim of this work was to evaluate the kinetic of PAH sequestration with the surfactant extraction method. The elaboration of a three-compartment kinetic model allowed the determination of rate constants for seven different PAHs in two natural sediments. The results suggest that dissolved [^{2}H]-PAHs are first adsorbed onto the surface of particles within a 4-7 d period as a function of their molecular weight or hydrophobicity and the sediment characteristics. Secondly, the adsorbed molecules became slowly sequestered and after a period of 17 d the sequestration process seemed to tend to an equilibrium with the surrounding environment.

In spite of their sequestration, PAHs may slowly desorb from the sediment particles as the time advances. Under this condition (long experiment), the equilibrium between adsorbed and desorbed PAHs, found also by Schuler et al. (2003) may be established. Therefore, aquatic organisms can bioaccumulate these toxic compounds with the result of a biomagnification in the food web. Thus, it is an important concern to evaluate the risk which PAHs can present to the environment after apparent sequestration. Further investigation will be needed to improve a kinetic model using a first-order consecutive reversible three-stage reaction on a long term experiment which may also help risk assessment of contaminated sites. Others factors have to be explored for a better understanding in the behaviour of sequestration process and surfactant in sediment matrix.

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Uranium in soil – an example with very skewed effect concentrations. S. Sheppard[1] and G.L. Stephenson[2]. [1]Ecomatters Inc., Pinawa, MB; and [2]Stantec Consulting Ltd., Guelph, ON.

Uranium is radioactive, but its half-life is so long that it presents a chemical toxicity rather than radiological hazard. The literature reports toxicity in soil at concentration as low as 0.5 mg/kg, and no effect concentrations above 10,000 mg/kg. Although the studies showing extreme sensitivity are not in top-line journals, there is no obvious reason to discredit them. The issue is important because Ontario has set their air quality guidelines on soil ecotoxicology. Additionally, CCME has set the guideline for U in agricultural soils close to background, and sufficiently low that remediation to that level would be extremely costly in areas such as Port Hope, Ontario. Previous Canadian studies showed that soil phosphatase was more sensitive than several other bioassays. A battery of other bioassays including seedling emergence (11 soils, 5 species), plant growth to maturity (2 soils, 2 species), legume nodule formation (2 soils), second-generation seedling emergence (2 soils), plant U uptake ratios (11 soils), 75 d earthworm survival (11 soils) and 75 d earthworm U uptake ratios (11 soils) showed few effects below 300 mg/kg. In 2003, further tests with soil bioassays formally developed by Environment Canada were undertaken, and soils with concentrations up to 3000 mg/kg were required to show a clear effect. The data from which U guidelines might be derived indicate a strong skew in reported effect concentrations, and this presents the assessor with an interesting dilemma.

Framework foundation and guidance for Tier 2 site-specific soil contact standards for PHC-contaminated sites. G.L. Stephenson[1], N.C. Feisthauer[1], M. Gilbertson[2] and J.H. McCann[3]. [1]Stantec Consulting Ltd., Guelph, ON; [2]Chris Wren and Associates Inc., Guelph, ON; and [3]Department of Biology, University of Waterloo, Waterloo, ON.
The management of petroleum hydrocarbons (PHCs) in soils in Canada is the result of the implementation of a three-tiered framework. Tier 1 involves the application of the Tier 1 national PHC soil standards that are considered to be protective of human health and the environment. Tier 2 involves the application of site-specific data or information to adjust the Tier 1 standards to determine Tier 2 levels that accommodate unique site characteristics. Tier 3 levels are generally derived from site-specific ecological or human-health risk assessments. The level of protection and the associated underlying guiding principles are preserved throughout this tiered process. However, it is recognized that the successive tiers represent increasing levels of precision, complexity, and cost. At a site where there is PHC contamination in soil and the Tier 1 standards exceeded for a particular fraction or fractions, then remedial action is required or, alternatively, a Tier 3 assessment might be undertaken. There is currently no mechanism or process to go from Tier 1 to Tier 2. Therefore, a project was designed to provide the foundation for a framework for the site-specific development of Tier 2 soil contact remedial standards for PHC-contaminated sites. An overview will be presented of the site conditions and factors that influence bioavailability of PHCs in soil; the role of sequestration in the bioavailability of PHCs in soil; and, the tools used to measure bioavailability of PHCs in soil.

Testing framework for assessing soils as part of a site-specific (ecological) risk assessment. G.L. Stephenson[1], N.C. Feisthauer[1] and M. Gilbertson[2]. [1]Stantec Consulting Ltd., Guelph, ON; and [2]Chris Wren and Associates Inc., Guelph, ON.
For most Tier 2 or Tier 3 site-specific ecological risk assessments, many of the toxicity data and much of the information on the chemicals of concern originate from the scientific literature. Site assessors appear reluctant to generate site-specific toxicity data, despite the advantages. Conducting single-species toxicity tests using a test battery of methods and species can generate data that reduce the uncertainty associated with the use of literature toxicity values and site-specific data reflect and integrate site-soil conditions and characteristics. To this end, an assessment framework has been proposed in order to address the risk associated with the soil contact exposure pathway. This exposure pathway is most critical for ecological receptors that live in or are closely associated with soil (plants, soil invertebrates, and burrowing mammals). The framework consists of a phased approach to testing. The first phase involves the use of acute screening and chronic tests with plants, soil invertebrates, and microorganisms exposed to undiluted soil samples from the site. The second phase of the testing framework also uses acute and chronic tests with these groups of test organisms but they invoke the use of soil dilution procedures or a contamination gradient to either identify threshold effect concentrations (acute/chronic) or to identify remedial targets (chronic). Different approaches to site soil assessment are used for different situations. The framework will be presented and these

approaches to soil assessment discussed as they relate to the assessment framework.

Valeurs de référence écotoxicologiques : du nouveau. A. Lafortune[1], A. Renoux[2], L. Martel[1], R. Chassé[1] et J. Trépanier[2]. [1]Centre d'expertise en analyse environmentale du Québec, Ministére de l'Environnement, Sainte-Foy, Qc; et [2]Sanexen Services environnementaux Inc., Varennes, Qc.
Dans le cadre d'un projet de recherche supportant la Politique de protection des sols et de réhabilitation des terrains contaminés, un protocole d'élaboration de valeurs de référence écotoxicologiques a été développé dans le but de générer de nouvelles valeurs à partir d'une revue exhaustive de la littérature. Les grandes lignes du protocole seront présentées, afin d'illustrer comment ces nouvelles valeurs ont été générées à partir des niveaux de protection et des récepteurs préconisés par la Politique, soit : revue de littérature, sélection des études pertinentes, montage de la base de données écotoxicologiques, calcul des valeurs de référence et établissement du niveau de confiance. L'utilisation d'un logiciel de gestion documentaire, d'une base de données électronique ainsi que le développement d'un logiciel de traitement des données se sont avérés nécessaires étant donné l'ampleur de la revue de littérature. Malgré cela, il n'a pas été possible de générer les valeurs de référence, nécessaires tant pour la validation des critères génériques que pour le calcul de critères spécifiques, pour 23% des substances initialement visées parmi celles de la Politique, soit parce que les données toxicologiques étaient inexistantes ou encore parce que les ensembles de données disponibles n'ont pu être utilisés pour générer des valeurs de référence. Grâce à ce projet de recherche, le Ministère dispose maintenant de nouvelles valeurs de référence, et ce, pour une cinquantaine de substances visées par la Politique.

Estimation of risk from bioassay data using the TerraSys software. A. Renoux et J. Trépanier. Sanexen Services environnementaux Inc., Varennes, Qc.
Even though risk assessment procedures based on the modeling of environmental conditions are widely used, mistaken conclusions can be drawn if information from the specific site is missing (i.e., lack of reference values or chemicals not characterized). For this reason, emphasis on the development and use of ecotoxicological bioassays was proposed during the last decade as a complementary tool for risk assessment to predict the hazard to biological receptors at a specific site. However, the ecotoxicological studies can generate bioassay data difficult to manage and to compare with risk estimates obtained with the more traditional modeling approaches. This presentation proposes a method of calculating risk indices from the results of bioassays for organisms in direct contact with soil. It has been designed so as to allow a direct comparison of the risk indices obtained from bioassays and from modeling and has been integrated into a software program for ecotoxicological risk assessment at contaminated sites, TerraSys v.1.0. This software supports the search for correlations between the bioassay results and the measured concentrations of chemicals on the site. The analyst may also use it to "integrate" the results of the two approaches, in such a way as to benefit from their complementary aspects, accounting for the strengths and weaknesses of each approach. A case study will be presented to illustrate the management of bioassay data with this method and the integrated assessment of risks with TerraSys.

The Sudbury soils study: a case study of using soil chemistry in ecological risk assessment. C.D. Wren[1], M. Butler[2], G.M. Ferguson[3], R.N. Hull[4] and G.D. Watson[5]. [1]Chris Wren and Associates Inc., Guelph, ON; [2]Falconbridge Ltd.; [3]Cantox Environmental Inc. Mississauga, ON; [4]Cantox Environmental Inc., Calgary, AB; and [5]INCO Ltd., Copper Cliff, ON.
A century of atmospheric emissions from mining and smelting operations in the Greater Sudbury Area has resulted in a significant amount of metal deposition in the surrounding landscape, particularly near

the three historic smelting and refining centres of Copper Cliff, Coniston and Falconbridge. In particular, the levels of Ni, Cu, Co and As are elevated in Sudbury soils. In 2001, approximately 12,000 soil samples were collected from a wide study area in the Sudbury basin and analyzed for a suite of metals and elements. This information is providing the base for a broad Human Health Risk Assessment (HHRA) and Ecological Risk Assessment (ERA) to examine the potential human and ecological risks associated with these historical and on-going smelter emissions. This presentation will demonstrate how the soil metals data are being incorporated into the ERA process. We will provide an overview of the spatial distribution of the chemicals of interest (COIs) in Sudbury soils, and how this pattern compares with current smelter emissions, as well as historical patterns of vegetation impacts. The large geographic scope of the study and volumes of background data contribute to the complexity of the undertaking. Environmental initiatives during the past two decades, including emission reductions and re-greening efforts, have resulted in improvements to the regional landscape and biodiversity. Thus, the ERA is being conducted during a period of ecosystem recovery. Scoping workshops have been conducted to help identify Valued Ecosystem Components (VECs) to be evaluated in the detailed risk assessment. The Sudbury Soils Study is one of the most comprehensive risk assessments conducted in Canada and includes examination of all environmental compartments including water, air, sediments, vegetation and dietary items in addition to soil.

Predicting the fate of fungi released into the environment using laboratory-contained intact soil-core microcosms. M. Providenti[1], S. Mautner[1], M. Smith[1], O. Chaudry[1], M. Bombardier[2], R.P. Scroggins[3] and E. Gregorich[4]. [1]Department of Biology, Carleton University, Ottawa, ON; [2]Environment Canada, Environmental Technology Centre, Ottawa, ON; [3]Environment Canada, Methods Development and Applications Section, Ottawa, ON; and [4]Agriculture Canada.

One of Environment Canada's obligation under the *Canadian Environmental Protection Act* is to determine whether substances on the Domestic Substances List (DSL) have the potential to be "toxic." Several commercially important fungi such as *Trichoderma reesei* are listed on the DSL, and methods to determine their environmental fate and ecological impacts are being developed. *Trichoderma* spp. are used extensively in industry and are routinely deposited into landfill sites as spent biomass from fermentation plants, but little is known about their ecological effects. We tracked the survival of a *T. reesei* strain over a six-month period in laboratory-contained, intact soil-core microcosms, a procedure designed to approximate natural field conditions within a controlled setting. We used quantitative polymerase chain reaction, a molecular procedure that allows the detection of specific genetic elements, to determine population levels of the fungus. Semi-quantitative viability testing was also carried out by dilution plating. Both procedures indicated that *T. reesei* abundance declined from initial inoculation levels to a steady-state after ~2 months. A 1 month long simulated winter period introduced into the experiment at ~4 months did not significantly affect *T. reesei* population levels. Based on these results, we predict that *T. reesei* strains will persist in soil for two or more seasons following release into the environment.

The effect of high and low dissolved oxygen on the toxicity of oil sands coke and its leachate to ***Chironomus tentans*****.** A.J. Squires and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Syncrude Canada Ltd. and Suncor Energy Inc. are currently mining the Athabasca oil sand deposits in northern Alberta. One of the waste products produced during the upgrading processes is coke. Coke is contaminated by various metals and organic compounds, which may have the potential to leach out after being exposed to water. The purpose of this study was to assess the effect of low dissolved oxygen on the long-term leaching potential of the toxic constituents found in coke. Coke from both companies was exposed to reconstituted water under low (0.9 mg/L) and high (7.9 mg/L) dissolved oxygen concentrations for a period of 30 d. During this time, samples of the overlying water containing leachate and the coke pore water were taken for chemical analysis. After the 30 d period, the benthic macroinvertebrate, *Chironomus tentans*, was exposed to both of the aged cokes and their overlying leachates. No significant ($p<0.05$) difference in survival or growth was found between the dissolved oxygen treatments, or among any of the leachate treatments. However, the *C. tentans* in aged Syncrude coke showed a significant ($p<0.05$) increase in growth. In contrast, Suncor coke significantly inhibited both survival ($p<0.005$) and growth ($p<0.05$) of *C. tentans* compared to the control. These results show that coke may have the potential to adversely affect benthic organisms if it is used in an uncovered capping option during an aquatic reclamation program.

Method summary of an echinoid embryo developmental test for determining the toxicity of sediment pore water. C. Buday. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Environment Canada's Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars), Report EPS 1/RM/27 December 1992, Amended November 1997, is a standard test used to monitor sediment toxicity. A few modifications to Environment Canada's Echinoid Fertilization test method allow the Echinoid Embryo Developmental test to be used to determine the toxicity of sediment pore water. Extending the sperm and egg exposure period from 10 min to 72 h allows the embryonic development of a fertilized egg to reach the pluteus stage and may provide a more sensitive test endpoint. A summary of the Echinoid Embryo Developmental test method and sediment pore water toxicity test results will be outlined.

A new tool for ecotoxicological risk assessment of contaminated sites: the TerraSys software. A. Renoux, J. Trépanier, P. Wallis, É. Richer, M. Cyr, M. Bisson, K. Morin et M. Fouchécourt. Sanexen Services environnementaux Inc., Varennes, Qc.

In order to respond to regulatory requirements regarding the management of contaminated sites and to facilitate the execution of ecotoxicological risk analyses, the company Sanexen has developed a software program called TerraSys. This program brings together an entire set of tools necessary for assessing the ecotoxicological risks of contaminated sites. It integrates various databases and numerous mathematical models for the estimation of contaminant concentrations in all environmental media, as well as the estimation of exposure levels and risk indices for representatives of the organisms living on the study site. It also proposes, as a complement to the traditional approach of mathematical modelling, a risk assessment approach using biotests conducted on samples taken from the site. The final risk interpretation step is then refined by integrating the results of the two approaches. The objective of this poster is to demonstrate the major characteristics of this new software and allow the reader to understand the process followed by TerraSys in assessing the ecotoxicological risks of contaminated sites.

Source to tap - tap to sewer / De la source au robinet - du robinet aux égouts

Session Co-chairs/Présidents: P.B. Jiapizian and/et T. Phommavong

Source water protection – it just makes sense, that's why! P.B. Jiapizian. Environment Canada, National Guidelines and Standards Office, Gatineau, QC.

Public health and water quality experts around the world agree that the multi-barrier approach (MBA) should be followed to ensure that drinking water is safe. The MBA consists of the management of the drinking water source, appropriate water treatment and management, and well-maintained and safe water distribution systems. As the first barrier of the MBA, protection of the source water is critical. It is important to evaluate all activities that threaten the quality of the drinking water source. Source water protection extends beyond controlling individual sources of contamination to address problems and solutions on a regional or watershed basis. Many provincial and territorial jurisdictions, as well as local governments, are already managing water quality programs with a watershed approach. However, drinking water programs heavily reliant on treatment technology to make water safe to drink have not always been involved in these efforts to protect or restore the quality of source waters. This is beginning to change. Source water protection is an effective, proven strategy for ensuring safe drinking water because of its high potential to be cost effective. A poor source of water can substantially increase the cost of treatment to make the water drinkable. When source water is so contaminated that treatment is not feasible, alternative water supplies can be expensive to obtain and cause delays in providing safe, affordable water.

The application of the CCME WQI as a performance measurement tool to assess the effectiveness of best management practices. R. Paterson, A. Ali Khan and H. Khan. Newfoundland and Labrador Department of Environment, Water Resources Management Division, St. John's, NL.

Source water protection is the first step in implementing the Multi-Barrier Strategic Approach for Drinking Water Safety. The goal of source water protection is to provide a multi-use watershed that can accommodate the activities of numerous stakeholders, while at the same time minimize the adverse effects on the environment. The Government of Newfoundland and Labrador has recognized the importance of source water protection since the early 1970s. The main objective of the program is to protect the water quality of drinking water sources through land use control within protected public water supply areas. A total of 262 public water supplies have been designated as protected areas under the program to date.

As a result of this program, many communities and private industries have worked together and committed to increased use of best management practices (BMPs) such as no development zones along water bodies, no development within sensitive areas, changes in traditional logging and other land use practices within protected water supply areas. BMPs have been implemented especially in watersheds with multiple land use activities and under pressure for development. The implementation of BMPs help to ensure that a balance is met in protecting environmental health, while at the same time encouraging local economic growth through resource development.

The Canadian Council of Ministers of the Environment Water Quality Index (CCME WQI) has been applied to drinking water quality data for one protected public water supply area to assess the water quality status with and without the use of BMPs. The case study of the Gander Lake Watershed demonstrated that the WQI can be used as a performance measurement tool to evaluate the effectiveness of BMPs. The strengths and shortcomings of applying the WQI were also discussed. Finally, the case study emphasized the need for the development of source water quality guidelines.

National Water Research Institute contributions in identifying threats to water quality (and enhancing the dialogue between scientists and decision-makers). A.T. Bielak. Environment Canada, National Water Research Institute, Burlington, ON.

Responding to growing concerns from Canadians about what is happening to their water supply, in 2001 scientists from Environment Canada's National Water Research Institute (NWRI) led a discussion of what they saw as the major threats to water quality in Canada. Workshop participants from various agencies and other research facilities agreed on a list of 15 major issues posing threats to sources of drinking water and aquatic ecosystem health. The resulting report describes each of these issues and identifies critical questions to be answered as well as the key challenges that researchers and governments will face in trying to resolve these issues. The workshop outcomes served to focus research priorities for NWRI and the Government of Canada, and provide essential information to guide policy and program development by various groups. Complementing the NWRI "Threats" report, the Institute is now also heavily involved in developing a second Threats assessment science document on threats to fresh water availability in Canada, as well as a number of science-policy initiatives with the CCME.

Cyanobacteria: the multiple-barrier approach to protecting the ecosystem and human health. T. Phommavong[1], M. Giddings[2], T. Macaulay[3] and M. Hilderman[1]. [1]Saskatchewan Environment, Environmental Protection Branch, Regina, SK; [2]Health Canada, Water Quality and Health Bureau, Ottawa, ON; and [3]Saskatchewan Health, Population Health Branch, Regina, SK.

Cyanobacteria, also known as blue-green algae, are naturally occurring contaminants of shallow, slow-moving, warm bodies of water, such as small lakes, reservoirs, sloughs and dugouts. They are commonly found on the Canadian prairies, but they are reported more often in other areas of the country. Although they have been observed for decades, it has been only in the last 10 years that authorities have become concerned about their potential health effects following long-term exposure. Increased eutrophication (nutrient loading of P and Ni) of susceptible water supplies has resulted in increased incidences of algal blooms. Cyanobacteria in a water source can be a considerable nuisance in the practical management of a reservoir, with problems ranging from increased biomass (affecting recreational use of the supply) to aesthetic problems (taste and odour complaints) to the development of toxins (cyanotoxins), which can be a concern for human health. While most species of blue-green algae are capable of producing various toxins (liver and nerve toxins being the most common), not all blue-green algal blooms produce toxins. When present, the amount of toxins can vary dramatically within the body of water and over time. Many of these toxins can adversely affect both aquatic species and human health. The multiple-barrier approach is "an integrated system of procedures, processes and tools that collectively prevent or reduce the contamination of drinking water from source to tap in order to reduce risks to public health." The presentation will focus on the use of the multiple-barrier approach in the management/control of the factors that affect bloom and toxin formation and the tools that can be used for protecting both the aquatic ecosystem and human health.

Watershed management and environmental quality objectives. H. Khan[1] and P.B. Jiapizian[2]. [1]Newfoundland Department of Environment, Water Resources Management Division, St. John's, NF; and [2]Environment Canada, National Guidelines and Standards Office, Gatineau, QC.

As the first barrier of the Multi-barrier approach, protection of the source water is critical. It is necessary to judge the effectiveness of protection measures undertaken, and whether the quality of source waters is adequate for the protection of human health. Currently, there are no guidelines for source water quality in Canada designed to protect human health before the water enters the treatment system. Environment Canada and the CCME are currently evaluating the feasibility of establishing a set of Environmental Quality Objectives (EQOs) specifically for source waters. Currently, some jurisdictions use differing thresholds in assessing source waters (i.e., drinking water guidelines) whereas others do not assess source waters continuously and are heavily reliant on treatment technology to make water safe to drink. These thresholds may be too conservative, given that the majority of source waters will undergo some form of treatment prior to distribution for human consumption. EQOs can be established limits or thresholds of biological and/or chemical contaminants in water set by governing bodies in order to ensure sustained protection of source waters for drinking water. These objectives may be narratives or numerical limits. EQOs should be set for hazards in source waters that are most commonly linked to hazards in the water supply system (e.g., turbidity, total organic carbon, microbiological pathogens). Only then will mitigative efforts to curtail hazards in source waters contribute to the overall reduction of risk to human health.

Probing the Ramsey Lake watershed. G.A. Spiers[1], A. Lock[1], D.A. Pearson[1], B. Hostetler[2] and J. Ray[3]. [1]Centre for Environmental Monitoring, Laurentian University, Sudbury, ON; [2]McQuarie University, Sydney, NSW Australia; and [3]Aquapath Canada Ltd., Temagami, ON.
The Ramsey Lake watershed, a "living laboratory" that has survived a century of extreme industrial acid and metal-laden emission insult, is at the centre of ongoing regional development, being a source for potable water, recreation and relaxation. With water sources being from groundwater, streams from conservation lands, and runoff from urban infrastructure, Ramsey Lake provides a unique challenge, responsibility and opportunity for environmental monitoring research to utilize both long-term and the real-time data from chemical and physical measurements of both inputs to, and exports from, the watershed. This presentation will highlight data obtained from both towed sensors measuring bottom water physical and chemical parameters, and from continuous monitoring equipment designed to allow development of dynamic predictive models crucial for planning and effective resource management. Instrumented buoys, profiling units and inflow samplers provide sub-daily limnological measurements that can be accessed remotely, in real-time, a feature particularly important for the detection of episodic events that may play a critical role in determining lake characteristics throughout the season. Analytical units providing data for wind speed, radiation, temperature, humidity and trace gas concentrations parallel the water quality monitoring initiatives. Innovative communication methods to water managers for this plethora of data will be outlined.

A fibre-optic probe for remote detection of *Eschericia coli* and total coliform bacteria in water. R.S. Brown[1], E.J. Marcotte[2], E. Lee[2], A. Ley[2], C. Gilmour[2], M. Brown[2], P. Aston[2], G. Cairns[2] and J. Poland[2]. [1]Department of Chemistry, Queen's University, Kingston, ON; and [2]Queen's University, Kingston, ON.
Eschericia coli monitoring in source waters, treated waters and waters in distribution systems is critical to ensuring the safety of a water supply in a multi-barrier approach. Current tests for *E.coli* and other coliforms require sample collection, transport to a laboratory facility, and then a 24 h incubation period to get a result, typically taking two days in total. We are developing a detection technology which is rapid and sensitive, yet simple enough to use in a remote and automated monitoring system. This will eliminate the need to transport samples to an external laboratory, providing same-day results at minimal cost. Our test is a modified version of standard *E. coli* tests using fluorogenic substrates such as 4-methyl-umbelliferyl-beta glucuronide (MUG). These "targeted substrates" detect the glucuronidase enzyme, the accepted indicator of *E. coli* in water samples. Our new system uses a custom designed substrate and a fibre-optic probe which detects only the product generated by the enzyme (with a parallel project for Total Coliform detection). Fluorescence is continually monitored by the instrument, providing the earliest possible indication of a positive sample. The probe can extract the optical signal even from samples which are coloured, turbid or have other fluorescent substances in them. Tracking signal over time allows estimation of the number of cells present in the initial sample, providing a quantitative test. Results of a pilot study on raw water samples will be discussed.

Designing the best strategy to manage municipal wastewater effluents. T.D. Ellison and C. Jefferson. Canadian Water and Wastewater Assoc., Ottawa, ON.
The traditional regulatory approach to managing wastewater effluents by senior levels of

government has tended to be focused on specific, individual substances that may have been found in the effluent streams. It has treated the wastewater treatment plant as the source of pollution and therefore the first line for enforcement. Acceptable concentration levels have been set based on aquatic toxicity testing (ignoring mixing zones), the effluent stream at the outfall has been monitored, and if the concentration found in the discharge exceeds the requirement, then the authorities proceed to prosecute.

Municipal wastewater systems (including stormwater systems) are simply a vector for the transport of pollutants that arise from other residential, institutional, commercial and industrial activities taking place in the municipality. The range of activities in municipalities varies enormously from system to system. The contaminants discharged into sewers and subsequently from treatment plants is largely unique to each municipality, and the receiving bodies of water are equally variable in their capacity to receive and assimilate or not assimilate the discharges.

Municipal authorities have limited powers to address the discharge of contaminated effluent streams into their sewer systems, and limited resources to institute source control and management programs. Many contaminants, once in the system are difficult to remove in conventional and even sophisticated treatment plants. They are therefore are likely to pass through these facilities in measurable quantities due to the sophistication of detection protocols. A new approach is needed that would recognize municipalities not as the first line of prosecutable persons, but as the first line of regulation and enforcement – a partner with the senior levels of governments in designing and implementing a wastewater management program.

This will involve strengthening the powers of municipal authorities to institute control instruments and then using them in concert with provincial and federal powers in a coordinated attack on the problems of pollutant generation and release from the true source of pollution – industrial and commercial activities. This paper will propose and expose the features of a trilateral, coordinated approach to effluent management in which the three levels of government are partners and not adversaries.

Metal bioaccumulation in caged mussels exposed to a municipal wastewater effluent. C. Gagnon[1], P. Turcotte[1], F. Gagné[1], C. Blaise[1] and M.H. Salazar[2]. [1]Environnement Canada, Centre Saint-Laurent, Montréal, Qc; and [2]Applied Biomonitoring, Kirkland, WA.

Urban wastewater discharges are important sources of contaminants such as metals to receiving waters. Physico-chemical conditions of the receiving waters influence metal speciation and bioavailability. The bioavailability and dietary uptake pathways of released metals in the receiving waters were investigated by an exposure experiment with caged mussels. Metal bioaccumulation was evaluated in tissues of the mussel specie *Elliptio complanata*, which was exposed for 90 d to the largest urban effluent discharging to the St. Lawrence River. Total and extractable particulate as well as dissolved and colloidal metal concentrations were also determined in surface waters of the effluent dispersion plume. Tissue distributions of certain metals (e.g., Ag, Cd, Cr) provided good tools to distinguish the exposure routes (dissolved vs particulate phase) for mussels exposed to municipal effluents and gills were generally the most important target tissues for metal bioaccumulation. Results of metal bioaccumulation showed that metals are generally less bioavailable in the effluent dispersion plume than at the reference site in the St. Lawrence River, and that environmental factors such as organic carbon concentration and the presence of colloids may control the metal uptake in the receiving waters.

Management of effluents from municipal waste water treatment facilities in Canada / Gestion des effluents des stations municipals de traitement des eaux usées au Canada

Session Co-chairs/Présidents: J.D. Clarke and/et B.A. Munson

The management of municipal wastewater effluents in Canada is changing - (the federal view). J.D. Clarke[1] and B.A. Munson[2]. [1]Environment Canada, Atlantic Region, Dartmouth, NS; and [2]Environment Canada, Prairie and Northern Region, Edmonton, AB.

As a result of requirements imposed by the CEPA 1999, and ongoing liabilities related to compliance with Section 36(3) of the *Fisheries Act*, Environment Canada is proposing some major changes to the way that municipal wastewater is managed in Canada. These changes include the development of a long-term strategy with a vision of no unacceptable risks from the release of wastewater effluents including treatment equivalent to secondary, water conservation and metering, and working with other levels of government to develop common parameters and limits for their discharge. The Canadian Council of Ministers of the Environment (CCME) is considering the development of a broad Management Strategy for municipal wastewater, using an environmental risk management model and a Canada-wide Management Strategy for implementation. As a first step in the federal long-term strategy, a Proposed Notice published on June 7, 2003 requires owners of specified wastewater facilities to prepare Pollution Prevention (P2) plans for ammonia, inorganic chloramines and chlorinated wastewater effluents. Following national consultations, the criteria for specifying facilities were modified significantly and now focus on effluent and receiving environment characteristics that reflect a higher risk of toxicity in the environment. Feedback from publication of the Proposed Notice is being reviewed for possible changes to the Notice. The federal implementation mechanism for the common parameters would likely be a *Fisheries Act* regulation. One of the most interesting aspects of this development work will be the interaction of the risk management and the more traditional end-of-pipe approaches.

A science based approach for the management of municipal wastewater discharges. C.B. Larose and L.A. Taylor. Environmental Services, Capital Regional District, Victoria, BC.

A wastewater and marine environment program (WMEP) has been conducted at the Capital Regional District's (CRD) Clover and Macaulay Point municipal wastewater outfalls in Victoria, British Columbia since 1988. The current program consists of monthly wastewater chemistry analyses, monthly surface water analyses for fecal coliforms, and a seafloor monitoring component. Seafloor monitoring includes sediment chemistry and benthic community analyses at Macaulay Point, and mussel tissue residue and population analyses at Clover Point. The primary goals of the WMEP are to ensure that wastewater discharges do not cause adverse effects in the receiving environment, to provide information to the CRD Source Control program, and to provide scientific guidance to wastewater managers. Monitoring results were used in the development of a process that provides an early warning of the potential for adverse environmental effects which allows for the timely development and implementation of appropriate source control and/or treatment alternatives. As part of this process, annual monitoring results will be compared to warning and seafloor trigger levels. A comparison to warning levels will determine the need for additional studies and/or more aggressive source control while exceedences of the seafloor trigger levels could result in treatment being implemented. Similar processes will be developed for the water column and sea surface waters off of both outfalls and will be used in conjunction with the seafloor component to manage Greater Victoria's wastewater discharges.

Derivation of a site specific ammonia limit for municipal treatment plant effluents. G.R. Craig[1] and I. Middelraad[2]. [1]G.R. Craig & Associates, Schomberg, ON; and [2]I. Middelraad & Associates, Guelph, ON.

Environment Canada is developing Environmental Quality Objectives to ensure that ammonia in effluents are not acutely lethal and concentrations in receiving waters are below sublethal effect levels. Ammonia acute lethality data for trout were reviewed for a size class of fish used in tests and a lower bound of LC50s under a range of Ph conditions was described. This produced an adjustment factor to be applied to a default un-ionized ammonia limit for different Ph conditions. The un-ionized ammonia limit was converted to total ammonia concentrations for a range of temperature and Ph conditions in an easy lookup format for plant managers. The site specific objective for trout was demonstrated to protect more sensitive species during short term exposures and would, under different receiving water Ph conditions, meet the PSL2 limit and CCME guideline for ammonia within 10-20 dilutions. This limit provides municipal plant operators a desktop approach to site specific ammonia management.

Redefining regulatory terms to develop rapid response action capability to protect ecosystems from biological contamination. A.J. Niimi[1] and V.G. Thomas[2]. [1]Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON; and [2]Department of Zoology, University of Guelph, Guelph, ON.

Regulations such as the *Fisheries Act* (1985) and the *Canada Water Act* (1985) include a number of provisions that were largely designed to protect aquatic ecosystems from chemical and physical impacts. These regulations are now facing challenges like protecting biodiversity in many water bodies and retaining the integrity of biological reserves. A major problem is the intentional, accidental and incidental introduction of new species that can undermine community structure and ecosystem stability. Amending existing legislation to include biological agents in the definition of a "pollutant" would be a positive step to deal with this issue. Extending the definition will be challenging because of the number of species involved, and development of concentration based criteria may not be suitable. Species that present a risk could be native or exotic to an area, and level of impact could be site specific. Water bodies impacted by chemical and physical agents can be rehabilitated through various action plans, but this may not be the case with a biological agent, therefore the cost of inaction can be large. Many natural resources agencies are currently developing Rapid Response Action Plans (RRAP) to deal with invasive species. Application of these plans to deal with a local problem is often limited because of the lack of legislative authorization. Examples will be presented of what agencies with RRAP capability and regulatory authority have done with the discovery of highly invasive species.

Microbial pollution: a key factor to consider in the management of municipal wastewater effluents from the "smallest" of sewage outfalls. J.F. Payne[1], L.L. Fancey[1], L. Park[1], C. Andrews[1], S.A. Whiteway[2] and B. French[3]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NF; [2]Jacques Whitford Environment Ltd., St. John's, NF; and [3]Oceans Ltd., St. John's, NF.

High volume municipal effluents may come under stringent regulations in the near future while small sewage outfalls dot the Newfoundland coastline. It has been established that sewage outfalls receiving effluents from populations as small as 50 people or less, have the potential to contaminate near shore intertidal and sub-tidal sediments to a considerable degree with various bacteria including *Clostridium*, total coliforms, fecal coliforms and *E. coli*. Studies were carried out at a number of sites including Harbour Grace, Harbour Breton and Carbonear Bay. Sediments from some small outfalls contained levels of *Clostridium* comparable to levels reported from a site in the United States receiving sewage from a population numbering in the hundreds of thousands. Studies in Harbour Grace and Carbonear Bay also indicated that sediments in deeper waters can act as reservoirs for high levels of *Clostridium* loading. Microbes (bacteria and viruses) in sewage may pose risks to the health of fish and marine mammals as well as human consumers of fish products. These results raise questions about the potential for cumulative impacts of clusters of small sewage outfalls versus single larger outfalls such as those in St. John's Harbour and Halifax Harbour. Studies have also been initiated in this regard on the health of fish around sewage outfalls.

Fate and effects of pharmaceuticals and EDCs: why the headache? / L'ABC des substances pertubatrices du système endocrinien et des produits pharmaceutiques

Session Co-chairs/Présidents: K.A. Kidd and/et V.L. Trudeau

Fish, frogs and pharmaceuticals in the aquatic environment. V.L. Trudeau[1], T.W. Moon[1] and C.D. Metcalfe[2]. [1]Department of Biology, University of Ottawa, Ottawa, ON; and [2]Environmental and Resources Studies Program, Trent University, Peterborough, ON.

Human and veterinary pharmaceuticals are now found in aquatic environments. These may represent an endocrine disruption and toxicological threat with a twist. For most drugs the mechanisms of action are well studied in mammals and some but not all of these cellular and biochemical pathways will be conserved in non-target aquatic species. We are currently examining the effects of steroids and lipid lowering drugs in the leopard frog and the goldfish. Waterborne ethinyl-estradiol(EE2), a contraceptive steroid, affects growth and development in tadpoles and regulation of gene expression in fish brain. In addition, EE2 causes feminization of gonadal development in the Japanese medaka, including complete sex reversal or gonadal inter-sex, depending on dose. The lipid lowering drug Gemfibrozil, a known peroxisome proliferators has multiple effects in fish by affecting growth hormone, lipid and carbohydrate levels and lowering sex steroid production. An overview of these results will be presented. How we should use the extensive mammalian pharmacological database to predict effects of pharmaceuticals on aquatic vertebrates will also be discussed. Supported by CNTC and NSERC.

Do pharmaceuticals in the environment get into non-target aquatic species? C. Mimeault[1], V.L. Trudeau[1], C.D. Metcalfe[2] and T.W. Moon[1]. [1]Department of Biology, University of Ottawa, Ottawa, ON; and [2]Environmental and Resources Studies Program, Trent University, Peterborough, ON.

The presence of pharmaceuticals in the aquatic environment is well established but the effects of these drugs on non-target species are only now being investigated. We previously demonstrated that, at therapeutic doses, gemfibrozil (GEM), a lipid lowering fibrate drug found in the environment, lowers triglycerides by over 50% in goldfish (*Carassius auratus*). GEM is known to be a peroxisome proliferator and increases the size and number of peroxisomes, the rate of β-oxidation and induces the formation of liver tumors in rodents through a nuclear receptor, the peroxisome proliferator-activated receptor (PPAR). We are now investigating the uptake route of GEM by goldfish. In order to do so, we exposed male goldfish to GEM by three different routes: (i) injections (0 and 10 mg/kg of body weight), (ii) diet (0, 0.5 and 10 mg/kg of food), and (iii) water (0, 1.5 and 10 mg/L) for 96 h. Blood samples were collected every 24 h and GEM plasma concentration detected using HPLC/MS analysis. In addition, biomarkers of exposure to peroxisome proliferators, including PPAR MRNA levels and peroxisomal activities will be assessed. Investigating the exposure route to GEM and eventually other pharmaceuticals, is key to understanding the environmental impact of drugs on non-target species and thereby increase the relevance of these studies. Funded by NSERC and CNTC.

QSAR based toxicity ranking and prioritization of ~3000 pharmaceuticals. H. Sanderson, K.R.

Solomon, R. Brain, D.J. Johnson, C.J. Wilson and T. Reitsma. Centre for Toxicology, University of Guelph, Guelph, ON.

Pharmaceuticals have been reported in surface waters, prompting legitimate public concern, as pharmaceuticals are biologically active compounds used daily by the public. Currently there are ecotoxicological data available for <1%, thus, the European Union Commission's scientific committee on toxicity, ecotoxicity and environment (CSTEE) recommended use of (Q)SAR models and precaution to prioritize further risk assessment of ~4500 compounds. In this paper we ranked 2986 different pharmaceutical compounds in 51 classes relative to hazard toward algae, daphnids and fish using the EPIWIN program. This ranking cannot be used to acquit compounds predict relative ranking in a situation were data is absent. Modifying additives were the most toxic classes. Cardiovascular, gastrointestinal, antiviral, anxiolytic sedatives hypnotics and antipsychotics, corticosteroid, and thyroid pharmaceuticals were the predicted most hazardous therapeutic classes. The overall relative order of susceptibility was estimated to daphnids > fish > algae. Expert judgment is needed to assess specific hazards for classes like microbial resistance and antibiotics, sex hormones and endocrine disruptors, etc. Accurate assessments of subtle effects of multiple pharmaceutical stressors on ecosystem health necessitate testing in microcosm. As human and ecological health are interconnected and subject to the precautionary principle, harmonization of evidence for correlation and causality of adverse effects seems sensible in an ethical and cost-effective context to facilitate substitution of hazardous compounds.

The tributyltin: a potential sex hormone disruptor in a *Mya arenaria* study in mesocosms. A. Siah[1], J.C. Pellerin[1], R. Saint-Louis[1], J. Amiard[2] et É. Pelletier[1]. [1]Institut des Sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, Qc; et [2]Service d Écotoxicologie, Université de Nantes, Nantes Cédex, France.

Abstract

An experiment carried out using mesocosms was conducted to assess the effects of TBT on reproductive sex steroid hormones. Flow-through exposure experiments of TBTCl at nominal concentrations 0, 1, 10 and 100 ng TBTCl/L were conducted on soft-shell clams, *Mya arenaria* from Anse à l'Orignal (Québec, Canada) during 5 July-10 October, 1999. In the organisms exposed to 10 ng TBTCl/L, we obtained an accumulation of 460 ng TBT as Sn/g gonad dry weight after two months of exposition. The level of TBT in the gonads of the organisms exposed to 100 ng TBTCl/L has increased from 41 ng in August to 1031 ng TBT as Sn/g gonad dry weight in October. In the both sexes, the level of progesterone did not differ between the organisms control and contaminated. Nominal concentrations of 10 and 100 ng TBTCl/L caused significant increase testosterone after one month of exposition and 17β-estradiol after three months of exposition in the gonads of exposed females. In the males, only the 17β-estradiol was significantly higher after three months of exposition to 100 ng TBTCl/L. Accumulation of TBT in the gonads of M. arenaria may disturb the normal pattern of testosterone and 17β-estradiol. Further studies was necessary to elucidate the mechanism of TBT action on the hormones production in the gonads of *M. arenaria.*

Keywords: *Mya arenaria*, tributyltin, endocrine disruptors, mesocosms, progesterone, testosterone, 17β-estradiol, histopathology.

Introduction

Tributyltin (TBT) has been used in marine antifouling paints as a biocide to prevent attachment and growth of organisms on the hulls of vessels (De Mora and Pelletier 1997). TBT has been considered as the most toxic substance introduced in aquatic environments (Goldberg 1986). Disruption of reproduction in gastropods, also known as imposex, can be caused by a few nanograms of TBT per liter of seawater (Gibbs and Bryan 1986). Accordingly, several countries such as France (1982), USA (1986), UK (1987), Canada, New Zealand (1989) and Europe (1991) have restricted the use of TBT to vessels less than 25 meters (Stewart and Thompson 1994). Regardless of the measures taken by legislation in respect to TBT, several studies have shown a persistent presence of TBT in the marine environment (Chau et al. 1997, Saint-Louis et al. 1997). Actually, it also known that concentrations of TBT may range from undetectable levels in open oceans, to 10 ng/L in estuaries and up to 100 ng/L in harbors (Oberdörster et al. 1998).

The effects of TBT on gastropods from the molecular level to the population impact give the best example of the effects of endocrine disruptor contaminants on marine invertebrates (Matthiessen et al. 1998). In female neogastropods, TBT causes the imposition of male characters such as development of a penis, vas deferens and seminiferous tubules (Gibbs and Bryan 1986, Gibbs et al. 1991). A result of imposex development can be sterility of the female population and thus leading to an eventual population decline (Matthiessen et al. 1998). Furthermore, studies on the veliger larvae of *Mytilus edulis* have revealed that TBTO was highly toxic even lower levels as 100 ng/L (Beaumont and Budd 1984).

In female snails, TBT disturbs the hormone metabolism by increasing the level of androgens (Matthiessen et al. 1998). Limited studies of effects of TBT on hormone metabolism in clams are available. The only research made on the clam *Ruditapes decussata* was done in the Mediterranean area (Morcillo and Porte 1997, Morcillo et al. 1998, Morcillo and Porte 2000). Their observations support the hypothesis of a masculinization of clams after TBT exposure (Morcillo and Porte, 2000) since increase in testosterone levels was observed in situ (Morcillo et al. 1998).

The St. Lawrence River, a major shipping lane for international containers, has been subjected to TBT contamination which has been recorded through the analysis of the River sediments (Regoli et al. 1999), zebra mussels (Regoli et al. 2001), sea stars (Pelletier and Normandeau 1997) and beluga whales (Saint-Louis et al. 2000). Soft-shell clams, *Mya arenaria*, which live buried in sediments, play a key role in the ecosystem of the intertidal zone biota of the St. Lawrence River (Desrosiers and Brêthes, 1984). These species are unable to metabolize TBT and can be useful bioindicators of mild to moderate concentrations of TBT contamination (50-1000 ng/L) (Kure and Depledge 1994). *M. arenaria*, a bioaccumulator of TBT, was able to concentrate TBT by a factor of 57 000 to 220 000 in Danish coastal waters (Kure and Depledge 1994), and has been shown to accumulate TBT in gonads up to 400 ng/g d.w. in the St. Lawrence River (Pellerin et al. 2002, Siah et al. 2003) or in mesocosms (Saint-Hilaire 1997).

The object of this study was to determine whether exposure of *M. arenaria* to TBT would lead to a disturbance in sex steroid hormone levels. It is worth noting that steroid hormones which regulate the gametogenesis cycle in *M. arenaria* exhibit seasonal variations, linked to sexual maturation (Siah et al. 2002). Therefore, it was necessary to understand the role of sex steroid hormones during the reproductive cycle of *M. arenaria* for a better interpretation of these variations. We have used experimental mesocosms to better understand the importance of environmental factors on sexual maturation of the soft shell clams and to bring evidence of a dose-response relationship between TBT and steroid hormones levels.

Materials and Methods

Work was done in experimental mesocosms located at the aquaculture station of ISMER, at Pointe-au-Père, Qc, Canada. Clams were exposed for a period of four months to 0, 1, 10 and 100 ng TBTCl per liter of seawater in large mesocosms containing 3000 L of filtered seawater (sea water). The experiment was conducted during 5 July–10 October, 1999 (acclimated 1 June–5 July). The water was contaminated with TBTCl (flow rate = 1 ml/min) in an open system. *M. arenaria* was collected at Anse à l'Orignal which is considered to be a reference site since no direct sources of pollution are found at this site. After sampling, the organisms were buried in sediments in containers. Each mesocosm contained three containers. Clams were sampled in each mesocosm (n=21). After rinsing clams with seawater, they were rapidly placed in coolers containing crushed ice and transported to our field laboratory.

Steroid extraction in the gonads of M. arenaria was performed using the method developed by Hines et al. (1990) and adapted by Siah et al. (2002). The recovery of progesterone was estimated to 65±9% after adding 55 pg of progesterone to homogenate. The recovery of testosterone from spiked homogenate with 100 pg of testosterone standard (n=4) was 83±12%, while recovery of 17β-estradiol with 100 pg of 17β-estradiol standard (n=3) was 76±9%.

Enzyme assay for progesterone levels of the gonads in males and females *M. arenaria* were quantified using ELISA Kits purchased from Cayman Chemical Co. (Ann Arbor, MI) (Siah et al. 2002). Standard curves were carried out with progesterone and 17β-estradiol standard concentrations between 0 and 1000 pg/ml and with testosterone between 0 and 500 pg/ml. Sample concentrations were extrapolated from these curves according to EIA Kits (Cayman Chemical Co). All samples and standards were prepared in duplicate.

The processing for TBT analysis was adapted from the method developed by Chau et al. (1997). The standard curve for the quantification of TBT was carried out with solutions of known concentrations of TBT standard between 0 and 0.5 μg/ml. The detection limit for TBT was 41 ng/g as Sn d.w. The recovery of butyltin species from spiked gonad tissues homogenate was 69±7.5% for TBT (n=6). TBT data have not been corrected for extraction recovery.

Statistical results of this study are presented as the mean of the samples collected each month. Sigma Stat for Windows (Jandel Corp.) was used for statistical analysis. A Student t-test for a normal distribution was used to compare two groups. When the distribution was not normal, a Mann-Whitney rank sum has been used. To assess multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data with a Turkey test. When the distribution was not normal, a Kruskal-Wallis one way analysis of variance on ranks has been used.

Results

Sediment temperatures in the mesocosms ranged from 6-12°C (Fig. 1). It is worthwhile mentioning that temperatures in the mesocosms were highest in the month of August (12°C) and the temperature dropped to 6°C in October. Organotin concentrations in the gonads of both the control and the TBT contaminated mesocosms are reported in Fig. 2. The concentration is represented in nanograms of Sn per gram dry weight of gonad tissue. TBT was detected (> 40 ng as Sn/g gonad d.w.) at all the gonad samples analysed except for July and the control in September. Concentration of TBT ranged from the detection limit 41 ng as Sn/g gonad dry weight in control organisms to 1031 ng as Sn/g gonad dry weight in the gonad of clams exposed to 100 ng of TBTCl/L sea water for three months. Lesser amounts of TBT (41 ng as Sn/g gonad d.w.) were detected in the gonad tissues of controls in August and October, in the clams exposed to 1 ng TBTCl/L sea water during the experimental period, in the clams exposed to 10 ng TBTCl/L sea water in October and in the gonads of the clams exposed to 100 ng TBTCl/L sea water in August. The highest value (1031 ng as Sn/g gonad d.w.) was measured for gonads of the clams exposed to 100 ng TBTCl/L sea water in October.

Steroid levels observations indicated differences in the levels of progesterone were not significant between the organisms control and contaminated for all the mesocosms and all the time of exposition. Variation of steroid levels in the gonads of clams exposed to 1 ng of TBT/L is reported in Fig. 3.

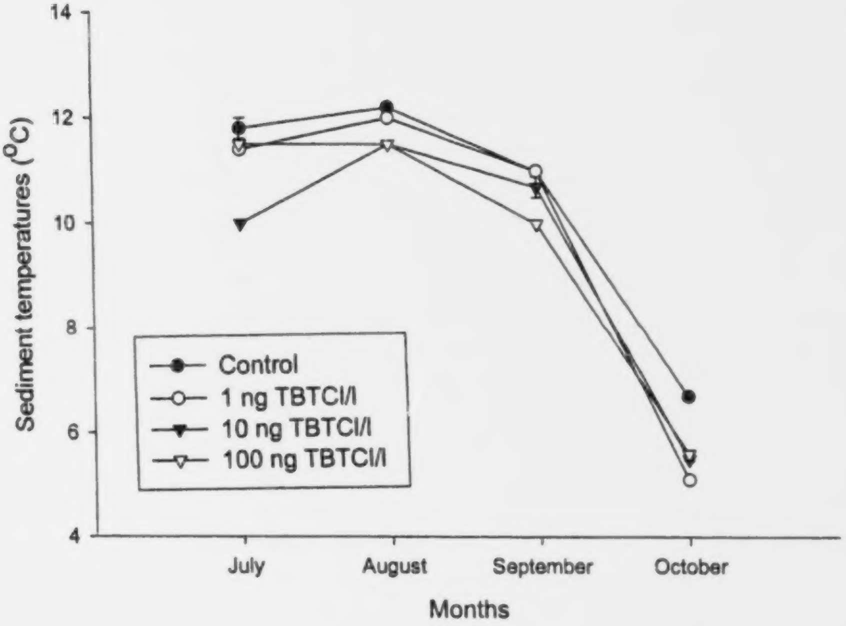

Fig. 1. Variation of sediment temperatures in mesocosms between July and October 1999. Each data point represents the monthly average of values recorded in each mesocosm.

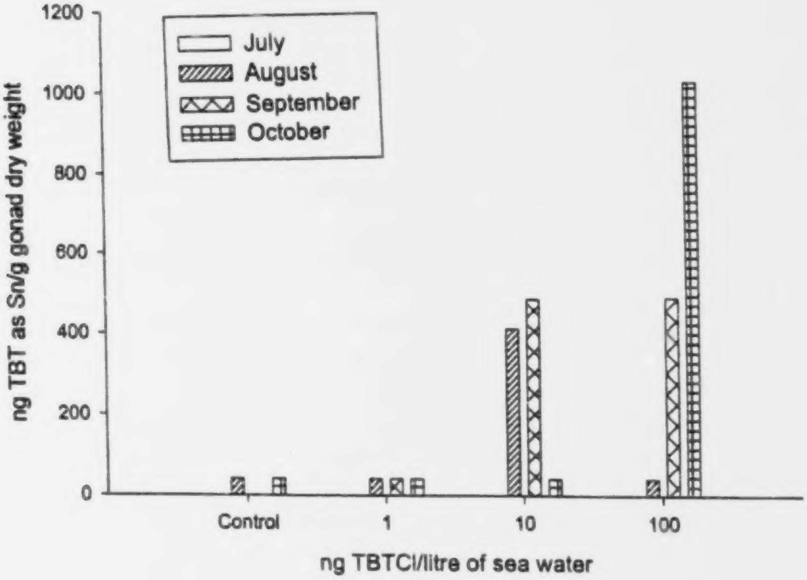

Fig. 2. The concentrations of TBT as nanograms of Sn per gram dry weight in the gonads of *Mya arenaria* of both the control and contaminated mesocosms.

Levels of testosterone in the female clams (66.4 pg/g) were higher than in the control (36.2 pg/g) in the month of September (two months after exposition to TBT). A testosterone level in males did not differ in comparison to the control. 17β-estradiol levels are more important in both females (175.6 pg/g) and males (115.6 pg/g) in the month of October in comparison to the control (47.3 in females and 46.4 pg/g in males).

Variation of steroid levels in the gonads of clams exposed to 10 ng of TBT/L sea water is reported in Fig. 4. Levels of testosterone were significantly ($p=0.001$) more important in the female gonads of clams exposed to TBT in August (104.3 pg/g) in comparison to the control (44.9 pg/g). Levels of 17β-estradiol were significantly ($p=0.01$) higher in October in females (372.8 pg/g) in comparison to the control females (47.3 pg/g).

Variation of steroid levels in the gonads of clams exposed to 100 ng of TBT/L is reported in Fig. 5. Progesterone levels are lower in July (388.3 pg/g) and August (555.2 pg/g) in females exposed to TBT in comparison to the control (895.9 in July and 1153.6 in August). However, the level of progesterone increased in September (961.4 pg/g) and more importantly in October (1822.1 pg/g) in comparison to the control (831.9 pg/g in September and 929.2 in October) but the difference was not significant. Testosterone levels were significantly ($p=0.01$) more important in the female gonads exposed to TBT in August (91.2 pg/g) in comparison to the control (44.9 pg/g). Finally, in October, the 17β-estradiol levels were significantly higher in females (177.3 pg/g, $P=0.001$) and males (130.8 pg/g, $p=0.01$) in comparison to the both female (47.3 pg/g) and male (46.4 pg/g) controls.

Discussion

Organotin pollution has been monitored in molluscs from the Catalan coast (Spain) and the Saint Lawrence River. TBT in the clam *R. decussata* from Mediterranean area was the major compound detected ranging from 200-1100 ng/g w.w. as Sn (Morcillo and Porte 1999). In the Saint Lawrence River, TBT concentrations in zebra mussels of the Québec City harbour area were between 37 and 1078 ng/g w.w. as Sn (Regoli et al. 2001) and TBT concentrations in the gonad of *M. arenaria* near the harbour of Rimouski were approximately 145 ng/g w.w. as Sn (Pellerin et al. 2002, Siah et al., 2003). In our study, we used mesocosm experiments to expose *M. arenaria* to different concentrations of TBT. Clams exposed to 0.1 and 10 ng TBTCl/L sea water exhibited an accumulation of TBT in their gonadal tissues ranging from 409 to 646 ng/g d.w. as Sn after two months of exposition. When *M. arenaria* was exposed to 100 ng TBTCl/l sea water, we obtained an accumulation of 491 ng/g d.w. as Sn in the gonad when exposed for a period of two months and of 1031 ng/g d.w. as Sn after three months of exposition.

M. arenaria exposed to TBT exhibited a significant change in gonad levels of testosterone and 17β-estradiol. Our mesocosm experiments have shown us increasing levels of testosterone by 50% in August following by an increase of 17β-estradiol between September and October in female gonads exposed to 10 and 100 ng TBTCl/l sea water as nominal concentrations in comparison to the controls. These observations suggest that the female gonads exposed to TBT showed an increase of testosterone which probably has been converted on 17β-estradiol by aromatase. This disturbance of the steroid hormones levels leaded to the consequences observed in sexual maturation. In the males, we observed only an increase of testosterone by 70% after exposition to 0.1 ng TBTCl/L sea water while 17β-estradiol level increase in the males exposed to 100 ng TBTCl/L sea water after three months (October). However, in July where the clams were not exposed to TBTCl, 17β-estradiol titres in the male gonads were higher in comparison to the control.

In TBT-polluted Mediterranean marina, *R. decussata* presents a 33% increase in testosterone titres in its tissues where TBT concentration was approximately 290 ng/g w.w. as Sn (Morcillo and Porte 2000). Conflicting data have been presented concerning the effect of TBT on 17β-estradiol levels. No

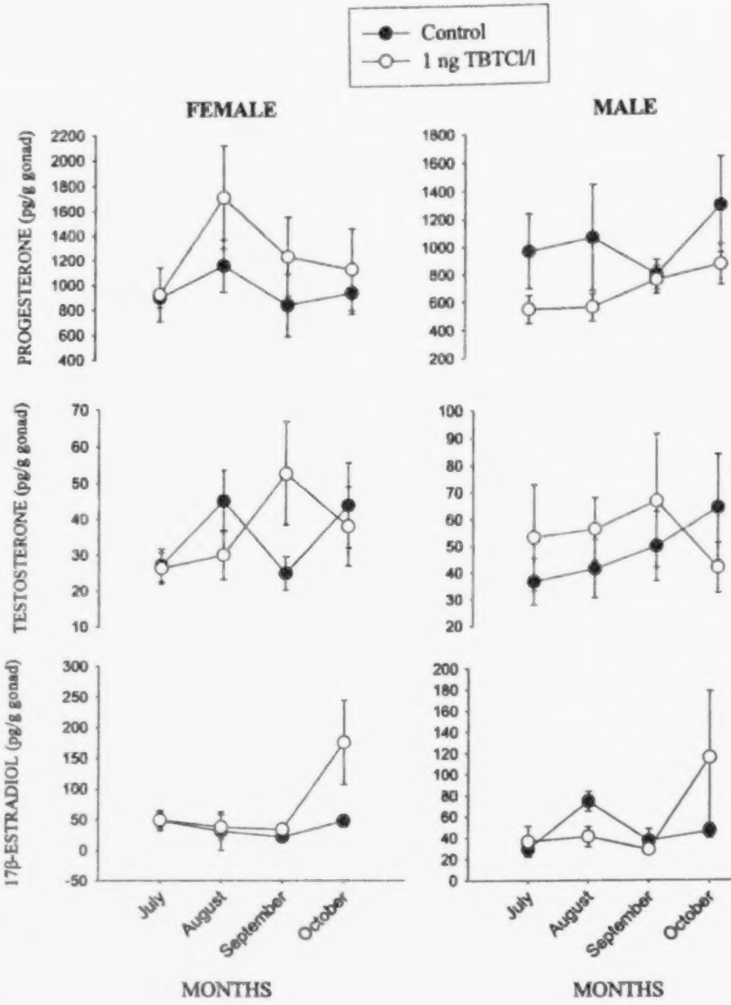

Fig. 3. Variation of progesterone, testosterone and 17β-estradiol levels in the gonads of males and females *Mya arenaria* sampled in both the mesocosm control and exposed to 1 ng of TBTCl/L. Each value indicates the mean±SE.

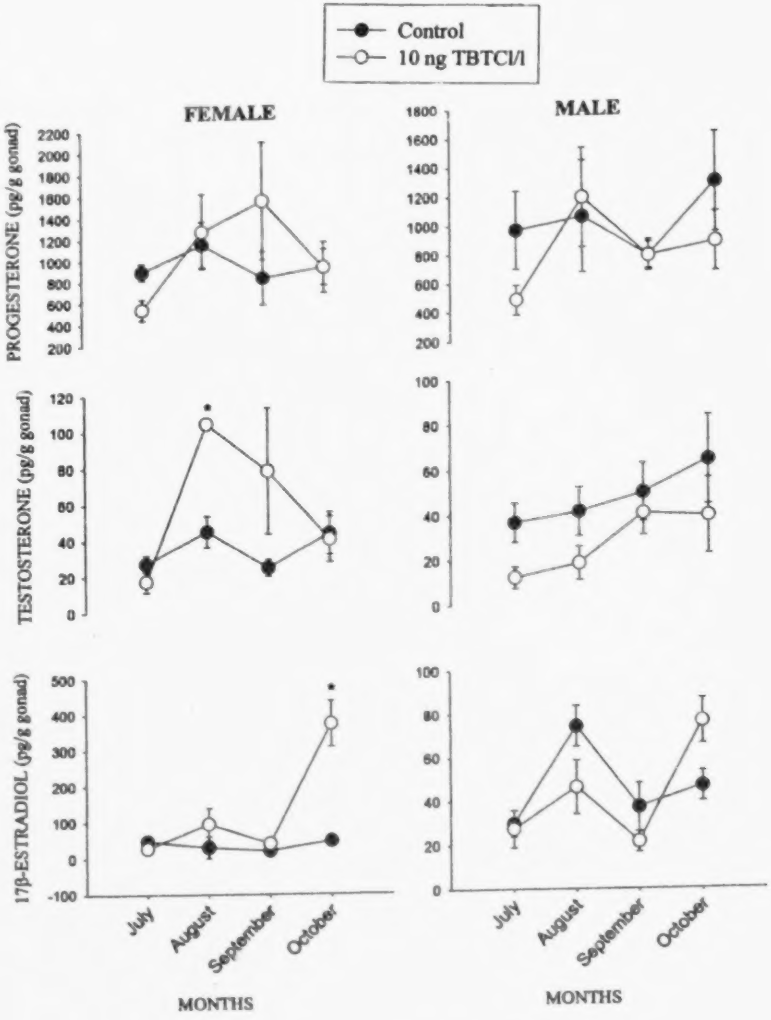

Fig. 4. Variation of progesterone, testosterone and 17β-estradiol levels in the gonads of males and females *Mya arenaria* sampled in both the mesocosm control and exposed to 10 ng of TBTCl/L. Each value indicates the mean±SE. Asterisks (*) indicate that value is significantly different to the control at P=0.05.

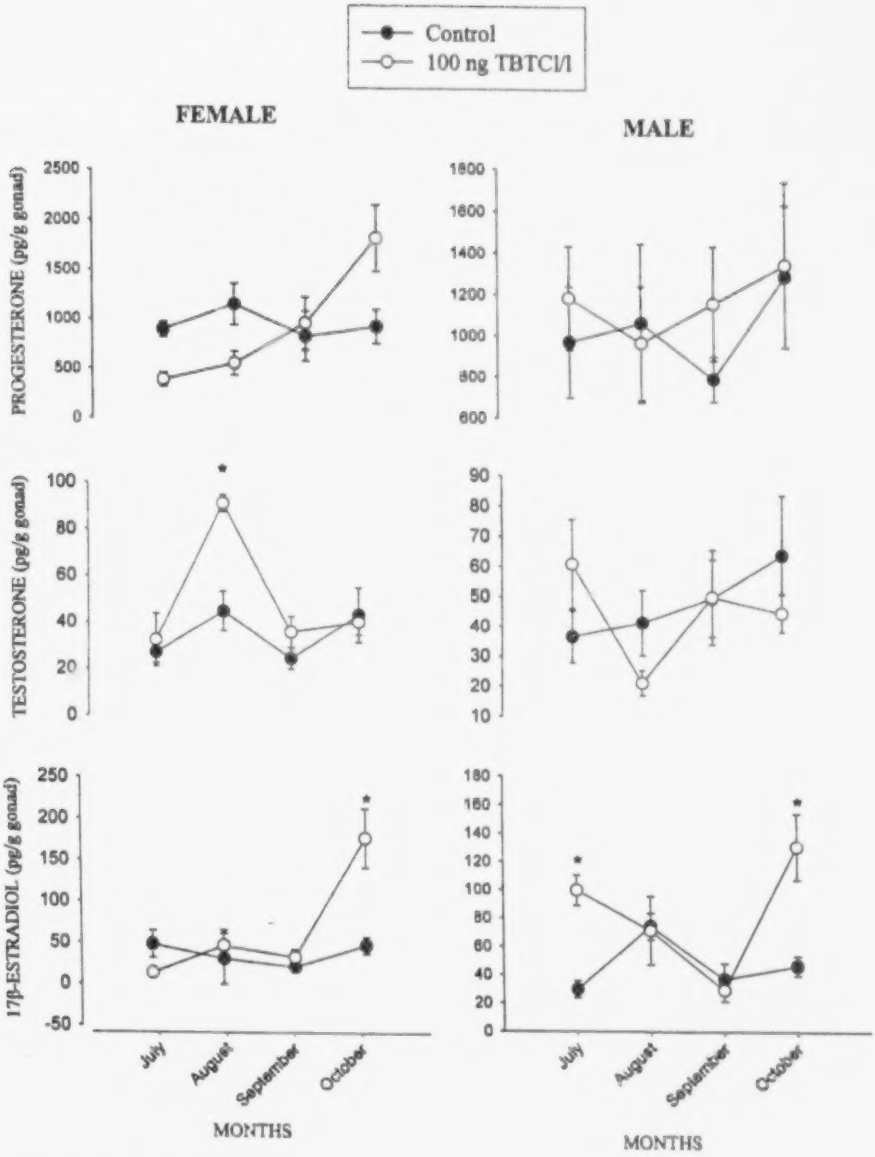

Fig. 5. Variation of progesterone, testosterone and 17β-estradiol levels in the gonads of males and females *Mya arenaria* sampled in both the mesocosm control and exposed to 100 ng of TBTCl/L. Each value indicates the mean±SE. Asterisks(*) indicate that value is significantly different to the control at P=0.05.

significant effect was seen in the formation of 17β-estradiol in the microsomal clams' *R. decussata* exposed to TBT (Morcillo et al. 1998). However, in the clams transplanted into a TBT-polluted marina, a depletion of 17β-estradiol levels was observed (Morcillo and Porte 2000).

In summary, in the mesocosm experiment, TBT accumulation has lead to a significant increase in testosterone in females and 17β-estradiol in both sexes but did not affect the amounts of progesterone in the gonads of *M. arenaria*. These observed hormonal change were more pronounced in females than in males. Our results suggest that the female gonads exposed to TBT showed an increase of testosterone which probably has been converted to 17β-estradiol by aromatase. However, the inhibition of the aromatase enzyme which converts androgens to estrogens has been anticipated as the causal mechanisms (Morcillo et al. 1998). Nevertheless, aromatase activity has never been directly determined in mollusc bivalve because the activity has been estimated for an amount of 17β-estradiol from 14C-testosterone source (Morcillo et al. 1998). Furthermore, the relationship between reduced aromatase activity and imposex symptoms is contradictory (Horiguchi et al. 2002). The mechanisms by which testosterone accumulates in the tissues of female *M. arenaria* can only be speculated. Studies have stipulated that TBT decreases the metabolic inactivation of testosterone as observed in *R. decussata* (Morcillo et al. 1998) and/or with the production of neurohormone by cerebral and pedal ganglia as shown in *Ilyanassa obsoleta* (Oberdörster and McClellan-Green 2000, Oberdörster and McClellan-Green 2002).

Overall, this work provides preliminary results on effects of TBT on sex hormone steroids of *M. arenaria* in mesocosm conditions. The obtained data demonstrate an increase of testosterone and 17β-estradiol levels in the female gonads of *M. arenaria* exposed to TBT with a damage of the germ cells in particular when the organisms were exposed to the concentrations of TBT founded in the harbour areas. TBT remains a threat to the reproductive endocrinology of benthic species as *M. arenaria* which are unable to degrade and eliminate this compound. Further research is required to determine exactly which mechanisms cause the observed hormonal change in the gonads of *M. arenaria*.

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Development of a bioassay to assess lethal and sub-lethal effects of pharmaceuticals on ***Hydra attenuata*****.** B.M. Quinn et C. Blaise. Environnement Canada, Centre Saint-Laurent, Montréal, Qc.
Pharmaceuticals enter the aquatic environment via treated sewage effluent, but their potential effects on non-target species remain largely unknown. Through a series of experiments a bioassay was developed to investigate the effects of several pharmaceuticals known to be environmental pollutants, using both acute lethal and sub-lethal responses in the freshwater cnidarian *Hydra attenuata*. Acute lethal toxicity was measured by determining 96 h LC50 values and developmental effects were appraised with a regeneration assay. Sub-lethal effects based on the microscopical examination of the morphology of each polyp following exposure and on a recently developed novel feeding test that measures the effect of exposure on prey (*Artemia salina*) capture and ingestion. Results provide relatively high LC50 values ranging from 1.3–2.2 mg/L for exposure to Loperomide, Carbamazepine and Ibuprofen, and ~22 mg/L for Acetylsalycylic acid and Diclofenac. In a preliminary feeding study using the reference toxin Cd, *H. attenuata* showed a significant reduction in prey ingestion at concentrations of around 0.12 mg/L, with ingestion providing more consistent results than prey capture. These sub-lethal endpoints proved to be more sensitive than the lethal endpoint. Results indicate that *H. attenuata* is a suitable species for investigating the effects of pharmaceuticals on freshwater organisms, offering simple, cost-effective bioassays for the study of both acute and sub-lethal toxicity.

Microcosm and laboratory studies into the effects of pharmaceuticals on freshwater plankton communities. C.J. Wilson, D.J. Johnson, H. Sanderson, K.R. Solomon and R. Brain. Centre for Toxicology, University of Guelph, Guelph, ON.
In a preliminary 35 d microcosm study, three widely used pharmaceuticals; Ciprofloxacin, Fluoxetine and Ibuprofen were examined as a mixture at four concentrations. The experiment showed a significant collapse of taxonomic diversity of zooplankton and phytoplankton compared to control. Abundance of zooplankton increased while phytoplankton decreased and then rebounded. In the laboratory, fluoxetine, an antidepressant, demonstrated acute lethality on *Daphnia magna*, (48 h LC50: 1100 mg/L), while Ibuprofen and Ciprofloxacin had no effect on this animal up to 10,000 mg/L. Evidence indicates that Ciprofloxacin is phytotoxic. Phytotoxicity of ciprofloxacin caused phytoplankton community affects. Fluoxetine affected some zooplankton but not others, allowing abundance increases. Similar antidepressant/SSRIs sertraline and clomipramine had effects comparable to fluoxetine, while effects of levofloxacin and sulfamethoxazole mirrored their antibiotic counterpart, ciprofloxacin. The effects of an 8-compound pharmaceutical mixture were tested in another microcosm study (acetaminophen, atorvastatin, caffeine, carbamazepine, levofloxacin, sulfamethoxazole, sertraline, trimethoprim). Abundance changes were evident over time in the treated groups compared to control, coupled with altered taxonomic composition. Lastly a single-pharmaceutical experiment was conducted with macrolide antibiotic tylosin. Preliminary results indicate increased algal growth due to treatment, with no effect on zooplankton density or diversity. Pharmaceuticals affect aquatic systems. Studies of direct effects indicate differential toxicity of the specific compounds. Current research aims to assess the functional as well as structural changes that

can occur as a result of a pharmaceutical perturbation.

Feminization of the FLFII strain of Japanese medaka (*Oryzias latipes*) **exposed to 17β-estradiol.** G.C. Balch[1], P.A. Wilson[2] and C.D. Metcalfe[1]. [1]Environmental and Resources Studies Program, Trent University, Peterborough, ON; and [2]Department of Biology, Trent University, Peterborough, ON.
The recently developed FLFII strain of Japanese medaka (*Oryzias latipes*) carries male and female specific DNA markers that allow for definitive identification of genotypic sex. Methods were developed to automate DNA extraction and profiling to facilitate the rapid determination of genotypic sex from caudal fin tissue. Comparison of the Y and X chromosome fragments indicates an 18 bp segment present in the Y-chromosome but absent in the X-chromosome. New primers were developed to include this deletion site. Automated profiling methods with 96-well plates permitted high throughput analysis of the genomic sex at rates of up to 500 fish per day. We investigated the sensitivity of the FLFII medaka strain to the potent estrogen, 17ß-estradiol (E2) and compared this to a widely used wild medaka strain (Carolina Biological Supply). In medaka exposed to 1 μg/L E2 from shortly after hatch to 100 d post-hatch, all FLFII medaka exposed to E2 (n=41) had the female gonadal phenotype. Among medaka from the wild strain exposed to E2, 47 of 48 fish had the female gonadal phenotype, indicating that the FLFII and wild strains have approximately equal sensitivities to the feminizing activity of E2. Molecular analysis of the FLFII indicated that 100% of the fish with the male genotype had been feminized to the female gonadal phenotype. These studies demonstrate that the FLFII strain is an excellent teleost model for detecting feminization or masculinization of fish and automated methods can be used for rapid analysis of the genotypic sex of FLFII medaka.

In Vivo **responses of rainbow trout** (*Oncorhynchus mykiss*) **exposed to two laundry detergent additives.** D.B. Simmons[1], S. O'Toole[2], G.C. Balch[2] and C.D. Metcalfe[2]. [1]Watershed Ecosystems Graduate Program, Trent University, Peterborough, ON; and [2]Environmental and Resources Studies Program, Trent University, Peterborough, ON.
Polycyclic synthetic musks are ubiquitous, and have been found in numerous river, sewage, lake and other water samples. Due to their lipophilicity (log Kows = 5.7-5.9), HHCB (1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-ã-2-benzopyran) and AHTN (6-acetyl-1,1,2,4,4,7-hexamethyltetraline) tend to accumulate in biological tissues. Gatermann et al., 1999, determined tissue levels of these polycyclic musks in Canadian freshwater fish ranging from 25-100 μg/kg lipid, or 3-9 ì/kg wet weight. (Chemosphere, **38**(14):3431-3441.). Our Data indicate that concentrations of polycyclic synthetic musks in the tissues of fish from the lower Great Lakes may exceed 1 mg/kg, depending upon sampling location and fish species. Schreurs et al. (2002) demonstrated that both HHCB and AHTN are selective estrogen receptor modulators (SERMs) *in vitro*, meaning that they can induce estrogenic and antiestrogenic activity (Toxicol. Appl. Pharmacol., **183**(1):1-9). In our evaluation of the *in vivo* responses of fish exposed to these synthetic musks, rainbow trout (*Oncorhynchus mykiss*) were injected intraperitoneally with the polycyclic musk compounds, HHCB and AHTN at a dose of 337.2 ìg/kg and 315.2 ìg/kg, respectively. After 5 d, trout were sacrificed and blood plasma and liver were collected for analysis of vitellogenin concentrations and EROD activity, respectively. Preliminary data with EROD indicate that the two test musk compounds do not induce hepatic EROD activity. Vitellogenin levels are being evaluated in the plasma of rainbow trout exposed to the test compounds. These data are relevant to assessing the potential for sublethal responses in fish that accumulate high concentrations of synthetic musks.

Pharmaceuticals in Canadian sewage treatment plant (STP) effluents: can they lead to reproductive impairment in non-target species? A.J. Woodhouse, T.W. Moon, V.L. Trudeau and

G.J. Van Der Kraak. [1]Department of Biology, University of Ottawa, Ottawa, ON; and [2]Department of Zoology, University of Guelph, Guelph, ON.

Gemfibrozil (GEM), a member of the fibrate class of drugs, is a peroxisomal proliferator and its presence has been detected in Canadian sewage treatment plant effluents. In preliminary studies we found that GEM injections resulted in decreased plasma sex steroid levels in the goldfish (*Carassius auratus*). Goldfish treated with 10 and 100 ìg GEM/g fish resulted in a 7% and 83% decrease in testosterone levels, respectively. The same treatments also resulted in a 15% and 56% decrease in 17β-estradiol levels, respectively. The mechanism(s) responsible for this decrease is being investigated. Two gonadal proteins, StAR (Steroidogenic Acute Regulatory protein) and PBR (Peripheral-type Benzodiazepine Receptor), are implicated in the transport of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where it is delivered to P450scc, the first committed enzyme of steroidogenesis. We have identified and partially cloned both StAR and PBR proteins in the gonads of the male goldfish (68% and 62% amino acid identity, respectively, to human equivalents). Molecular studies are currently underway to determine if GEM impacts StAR and/or PBR gene expression and steroidogenesis.

Determination of *in vivo* endocrine effects in fish from nonylphenol ethoxylates and their biodegradation metabolites. G.C. Balch[1], C.M. Foran[2], B. Peterson[3], E. Mihaich[4] and C.D. Metcalfe[1]. [1]Environmental and Resources Studies Program, Trent University, Peterborough, ON; [2]Department of Biology, West Virginia University, Morgantown, WV; [3]Department of Biology, University of Mississippi, University, MS; and [4]Rhodia Inc., Raleigh, NC.

Previous studies have demonstrated the endocrine disrupting potential of selected alkylphenols, however, industrial and environmental samples often contain mixtures of alkylphenol ethoxylates and associated biodegradation metabolites, of which relatively little is known. Many studies also report only nominal, not measure concentration, which make comparisons to measured environmental samples difficult. Japanese medaka(*Oryzias latipes*) were exposed under static conditions with a complete renewal of exposure water occurring three times per week. Exposures began one day after fish hatched and continued for the next three months. Nonylphenol and ethoxylate mixtures NP1EO, NP4EO, NP9EO plus the nonylphenol monoethoxycarboxylate NP1EC were tested at varying concentrations. Water samples were collected at selected times to determine degradation rates and hence averaged measured exposure concentrations. A total of 50 fish per exposure treatment were examined for mixed gender secondary sex characteristics (MSSC), histologically assessed for endocrine related alterations to gonadal tissues and assessed for vitellogenin in blood plasma. Nonylphenol tested at 29 µg/L (measured) was highly estrogenic with 82% of the males exhibiting gonadal intersex (testis-ova) and 42% of fish exhibiting MSSC. Nonylphenol at 9 µg/L was still estrogenic but only 5% of the males exhibited testis-ova and 20% of fish had MSSC. A weak estrogenic response was observed with NP1EO at 105 µg/L in which 22% of the fish exhibited MSSC while none in this treatment exhibited intersex. Estrogenic responses were not observed with NP1EC (2010 µg/L), NP4EO (380 µg/L), or NP9EO (540 µg/L).

In vivo **estrogenicity of nonylphenol, nonylphenol-1-ethoxylate and nonylphenol-1-ethoxycarboxylate using vitellogenin induction in juvenile rainbow trout** (*Oncorhynchus mykiss*)**: a re-evaluation of nonylphenol and its ethoxylates in the Canadian environment.** É.B. Dussault[1], J.P. Sherry[2], H.-B. Lee[2], B.K. Burnison[2] and M.R. Servos[3]. [1]Centre for Toxicology, University of Guelph, Guelph, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; and [3]Canadian Water Network, University of Waterloo, Waterloo, ON.

Nonylphenol polyethoxylates (NPEOs) are widespread chemicals used as nonionic surfactants. The presence of these polyethoxylates and their degradation products in aquatic systems has been well documented. The potential of these contaminants to disrupt endocrine function has been demonstrated, although the level of estrogenicity of nonylphenol and its ethoxylates is still debated. The relative estrogenicity of nonylphenol and its ethoxylates was investigated in a 21 d flow-through rainbow trout (*Oncorhynchus mykiss*) exposure to nominal doses of 1, 3, 10, 30, or 100 μg/L nonylphenol (NP), nonylphenol 1-ethoxylate (NP1EO), and 10, 30, 100, 300 or 1000 μg/L nonylphenol 1-ethoxycarboxylate (NP1EC). All three chemicals triggered plasma vitellogenin (Vg) induction, their relative estrogenicity being NP > NP1EO > NP1EC. Measurements of the relative potency of NP1EO and NP1EC, compared to NP, yielded ratios of 0.22 and 0.03, respectively, values in general agreement with relative toxicity data. A re-evaluation of the estrogenicity of the biodegradation products of nonylphenol polyethoxylates in Canadian sewage treatment plant effluents was performed, using the relative estrogenicity ratios determined in this study, and revealed that the added contribution of nonylphenol polyethoxylates to effluent estrogenicity is relatively minor. Indeed, adding the contribution of NP1,2EO and NP1,2EC resulted in concentrations of all three chemicals above the 1 μg/L ENEV threshold in less than 14% of Canadian STP effluents. However, given possible additivity with other potent compounds, any further contribution to effluent estrogenicity remains significant.

An ELISA for cutthroat trout (*Oncorhynchus clarki*) vitellogenin (Vg) and its use to assess the estrogenic impacts of agricultural runoff in a British Columbia watershed. J.P. Sherry[1], T. Hooey[1], S. Sylvestre[2], M. Sekela[2], T. Tuominen[2], P.D. Hansen[3] and B. Hock[4]. [1]Environment Canada, National Water Research Insitute, Burlington, ON; [2]Environment Canada, Aquatic and Atmospheric Sciences, Vancouver BC; [3]Institute of Ecology, Technical University of Berlin, Berlin, Germany; and [4]Department of Botany, Technical University of Munich, Freising, Germany.

We developed an enzyme linked immunosorbent assay (ELISA) for the measurement of vitellogen (Vg) in the plasma of cutthroat trout (*Oncorhynchus clarki*). The ELISA is based on a monoclonal antibody that was previously produced against rainbow trout (*Oncorhynchus mykiss*) Vg (Marx et al., 2001 Chemosphere **44**: 393-399). A triple precipitation procedure which was followed by column chromatography on DEAE Sephacel was used to purify Vg from the plasma of female cutthroat trout that had been induced with 17β-estradiol. The purity of the Vg was assessed by gel electrophoresis and Western Blots. The ELISA was calibrated, optimized, and characterized. The ELISA was used to measure Vg in the plasma of juvenile cutthroat trout that were exposed to surface water at a series of sites along an intensively farmed BC watershed. We used a flow-through regime to expose the fish to surface water. The exposures were long term (60 d) and the study was repeated over several seasons.

Assessing effects of pharmaceuticals on the aquatic environment in Canada: overview of approaches and assessment tools. K.R. Solomon[1], H. Sanderson[1], P. Sibley[1] and S.A. Mabury[2]. [1]Centre for Toxicology, University of Guelph, Guelph, ON; and [2]Department of Chemistry, University of Toronto, Toronto, ON.

The potential environmental effects of pharmaceuticals released into aquatic ecosystems are typically derived from single-species, acute laboratory bioassays on single compounds. However, exposures from human and agricultural inputs are usually chronic in nature and are invariably to mixtures. Microcosm bioassays allow the detection of direct or indirect effects of acute and chronic exposures to a large number of species at the same time and under more environmental realistic conditions. In several studies, probabilistically derived combinations of concentrations of mixtures and single pharmaceuticals were used to chronically (35-60 d) expose aquatic organisms in 12,000 L microcosms. Effects on phytoplankton, zooplankton, macrophytes, and fish were assessed over time through regular sampling. Plants (*Myriophyllum* sp., *Lemna gibba*) showed severe growth inhibition in the greater concentrations of some antibiotics as compared to controls. Further laboratory tests on *L. gibba* indicated that ciprofloxacin and a number of antibiotics can have adverse and additive effects. However, other pharmaceuticals were stimulatory. Effects on zooplankton and phytoplankton resulted in changes in structure and diversity of the system, possibly through treatment-related selective effects in these groups of organisms. The principles behind the use of microcosms and the assessment of mixtures of pharmaceuticals in these systems will be discussed and some illustrative examples of the effects on organisms in the above trophic levels will be presented. These studies will be placed in a larger context of assessing risks from these substances in the Canadian environment.

The use of genetic markers of frog metamorphosis for the detection of disruption of thyroid hormone action by acetochlor and sewage treatment plant effluent. C.C. Helbing[1], F. Zhang[1], L. Ji[1], N. Veldhoen[1], K. Ovaska[2] and G.C. van Aggelen[3]. [1]Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC; [2]BioLinx Environmental Research Ltd., Sidney, BC; and [3]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.
A wide variety of environmental contaminants have been shown to interfere with hormone signaling in vertebrates. However, relatively little is known about the effects and identities of chemicals that target thyroid hormone (TH) action, particularly in complex mixtures. Using TH-dependent frog metamorphosis and quantitative real-time polymerase chain reaction (QPCR), we examine the effects of the preemergent herbicide, acetochlor, and sewage treatment plant effluent on the expression of TH receptor genes in brain and tail tissues. We are able to detect perturbations in the expected TH responsiveness of these genes in brain and tail tissue indicating a disruption of TH signaling within 72 h of exposure. Exposure to acetochlor also changes the swimming behaviour of tadpoles suggesting that gene expression markers may be a useful predictor of perturbations in brain function. Therefore, using the frog metamorphosis model combined with genetic approaches may be an important tool for understanding the broader implications of disruption of thyroid hormone action.

Effects of xenoestrogens in the St. Lawrence River on male spottail shiner (*Notropis hudsonius*) **reproduction.** D.G. Cyr, J. Aravindakshan et V. Paquet. Institut National de la Recherche Scientifique - Institut Armand Frappier, Montréal, Qc.
We have shown that there is an important estrogenic contamination of the St. Lawrence River around the Island of Montreal. Our objective was to determine the effects on this contamination of male reproduction in spottail shiners (*Notropis hudsonius*). Histological staging of spermatogenesis (Stages 1-6) in shiners captured at a control site and at sites where fish are exposed to environmental estrogens was done. In control shiners, 95% of the fish had testis of either stage 4 (50%) and 5 (45%) of spermatogenesis. At Ile Dorval, where VTG mRNA levels are moderate, fish had testes of stage 3 (38%) and 4 (45%) and only 15% of fish were at stage 5. In contrast, at Ilet Vert and Beauregard, where VTG mRNA levels are high, none of the fish were at stage 5. The majority of fish were at stages 3 and 4 of development and almost 10% of fish were at stage 2. These data indicate that spermatogenesis is markedly decreased in xenoestrogen-exposed fish. Intersex was also determined. At our control site, a single fish had intersex (0.027% of fish sampled). At Ile Dorval, 15% of fish had intersex gonads, while 31% of fish at Ilet Vert and 27% of fish at Beauregard had intersex thereby indicating that testicular development is altered in xenoestrogen-exposed fish. Supported by CNTC.

Assessment of wild fish from some Canadian areas of concern for reproductive alterations. M.E. McMaster, G.R. Tetreault, C. Boyko and T. Janoscik. Environment Canada, National Water Research Institute, Burlington, ON.

Environment Canada has undertaken studies in Canadian Areas of Concern (AOCs) to determine the current state of fish and wildlife health. Phase One (2001-2005) is focusing on conditions in AOCs of the lower Great Lakes. The studies presented here will focus on AOCs in western Lake Erie, and the Detroit and St. Clair Rivers. Two different fish species were collected from exposed and reference sites and examined for reproductive health. As part of this study our laboratory measured circulating levels of reproductive sex steroids, the production of these steroid using an *in vitro* gonadal incubation assay as well as gonadal histology for differences in gonadal development due to exposure. These reproductive endpoints were compared to other indicators of overall fish health as well as other endocrine endpoints including thyroid function and circulating vitellogenin levels. This study is ongoing and its overall goal is to reassess all of Canada's AOCs for evidence of endocrine disruption.

An accumulation model for investigating active substances bioavailable to fish exposed to pulp mill effluents. L.M. Hewitt[1], A. Pryce[2], R. Schryer[3], B.K. Firth[4], A. Belknap[1], K.R. Munkittrick[5] and G.J. Van Der Kraak[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Zoology, University of Guelph ,Guelph, ON; [3]Golder Associates, Saskatoon, SK; [4]Weyerhaueser Ltd., Federal Way, WA; [5]Department of Biology, University of New Brunswick, St. John, NB.

We have been investigating the characteristics of bioactive substances present in effluents from pulp and paper mills across Canada and have developed a bioaccumulation model to study only those compounds bioavailable to fish. At a bleached kraft mill and a bleached sulfite/groundwood mill naïve male white sucker (*Catostomus commersoni*) were exposed to final effluent in controlled flow-through conditions. At an additional bleached kraft mill, wild sucker of both sexes were collected from the receiving environment and examined for the presence of bioactive substances. At each mill studied, hepatic tissues were extracted and lipid-free extracts were fractionated according to octanol-water partition coefficient (Kow). Fractions were tested *in vitro* for the presence of compounds functioning as ligands for: (i) the Aryl hydrocarbon receptor (AhR) using mixed function oxygenase (MFO) induction in H4IIE cells, (ii) the estrogen receptor (ER) isolated from rainbow trout (*Oncorhynchus mykiss*) liver, (iii) the androgen receptor (AR) isolated from goldfish (*Carassius auratus*) testes, and (iv) sex steroid binding protein (SSBP) isolated from goldfish plasma. At each mill, fish accumulated compounds from final effluents with the ability to interact with sex steroid hormone receptors, indicating potential effects on hormone signalling and transport. For the wild fish study, females showed significant hepatic elevations of bioactive substances, demonstrating gender differences of accumulations under environmentally relevant exposure conditions. The results to date show no correlation of accumulation of these compounds with effluent treatment and pulp production type. These findings are consistent with separate effluent evaluations of sex steroid receptor ligands before and after treatment from a larger set of 13 mills across Canada.

Responses of a freshwater food web to synthetic estrogen additions. K.A. Kidd[1], C.L. Podemski[1], M.J. Paterson[1], A.G. Salki[1], D.L. Findlay[1], V.P. Palace[1], P.J. Blanchfield[1], K.H. Mills[1], K. Liber[2], M.E. McMaster[3], R.E. Evans[1] and B.J. Park[1]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [3]Environment Canada, National Water Research Institute, Burlington, ON.

The potent estrogen mimic used in birth control pills has been added continuously over three summers (2001-03) to a lake at the Experimental Lakes Area in northwestern Ontario. Effects on the microbial through fish populations have been assessed in this lake during the estrogen additions, and contrasted to pre-addition and reference lake data. Male minnow species exposed to the environmentally-relevant concentrations of 17α-ethynylestradiol (EE2; 5-6 ng/L) produced high concentrations of the egg protein precursor vitellogenin, displayed impacted *in vitro* steroidogenesis, and have developed gonadal abnormalities (including intersex in one species). Female fish are being impacted at the biochemical through tissue level with increased production of vitellogenin and delayed development of oocytes. Population monitoring indicates that the abundance of the fathead minnow has decreased in the lake in response to EE2 additions; no changes in the abundances of the other three fish species (lake trout, white sucker, pearl dace) have yet been observed. Some impacts on lower-trophic-level organisms have also been found. For example, both the diversity (but not total abundance) of the algal community and the production of eggs by several species of zooplankton have decreased during EE2 additions. Intersex has also been observed in mink frog tadpoles (5.5 and 12.5% in 2001 and 2002, respectively) caged in the experimental lake but not in the reference systems. Low concentrations of a potent estrogen mimic can have adverse impacts on both vertebrates and invertebrates in aquatic ecosystems either through direct or indirect effects.

Short-term exposure to 17 α-ethynylestradiol alters normal development of testes in *Xenopus laevis*. S.M. Ruby[1], E. McKinley[1], C. Dimacacos[1] and M. Fournier[2]. [1]Department of Biology, Concordia University, Montreal, QC; and [2]Institut National de la Recherche Scientifique, Pointe Claire, Qc.

The synthetic estrogen 17 α-ethynlestradiol (EE2) is a commonly used oral contraceptive. It has recently been detected in sewage effluents. *Xenopus laevis* tadpoles were exposed for 48 h during sexual differentiation to 5 ng/L EE2 under static laboratory conditions at 21±0.5°C. Tadpoles were fixed and the kidney-gonad complex was microdissected and prepared for histological analysis. Testicular degeneration increased significantly from 16.7-85.7% in control and EE2 exposed testes respectively following a 48 h exposure period. Primary spermatogonia decreased significantly from 31.08±10.2 to 26.6±3.0. There was no significant differences in testicular lengths. The results suggest that short-term exposure to EE2 during sexual differentiation can significantly affect normal development of the testes for the life of the organism.

Toxicity testing of a polybrominated diphenyl ether (PBDE) commercial flame retardant using the *Xenopus* tail regression model. G.C. Balch, S. McDowell, K. Reiber and C.D. Metcalfe. Environmental and Resources Studies Program, Trent University, Peterborough, ON.

A commercial brominated flame retardant (BFR) was assessed for thyroid disrupting potential using the African clawed frog (*Xenopus laevis*) tail regression model. A primary constituent in this commercial BFR mixture (DE-71) was the pentabromodiphenyl ether congener 99 (2,2',4,4',5-PeBDE) which is frequently found in biota. Disruption to the circulating concentration of thyroid hormones (THs) can influence the rate at which the tail regresses and hence can be used to indirectly identify xenobiotics that have the potential to alter the production, concentration or signaling of THs. Three routes of exposure were assessed including (i) aqueous exposure, (ii) intraperitoneal injection (i.p.), and (iii) incorporation of the BFR into food. The greatest responses were seen when tadpoles were fed BFR amended food for a period of eight weeks, beginning at 1 week of age. A concentration of 1 mg DE-71 per gram of food significantly inhibited tail regression and metamorphosis. Higher concentrations (5 gk/g) resulted in reduced skin pigmentation and an even greater retardation of tail regression. Aqueous exposure protocols (up to 500 µg/L) starting at stage 58 failed to inhibit tail regression and although i.p. injections (60 µg/tadpole) at stage 58 did inhibit tail regression, the i.p. results were not as dramatic as those seen with the feeding trials. This work demonstrates the thyroid disruptive potency of a commercial BFR and is suggestive that critical windows of sensitivity occur at developmental stages earlier than stage 58, a time at which many tail resorption assays begin exposure.

Alkylphenols, estrogenicity and androgenicity in the Miramichi River, New Brunswick. D.T. Bennie[1], B.K. Burnison[1], C.A. Sullivan[1], S.B. Brown[1] and W.L. Fairchild[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB.

Declining Atlantic salmon (*Salmo salar*) catches in Atlantic Canada prompted studies into contributing factors. It has been hypothesized that the historical use of the endocrine disrupting substance 4-nonylphenol as a solvent in the formulation of Matacil 1.8D used to control spruce budworm infestations may have negatively affected the smolt stage and subsequent catch of returning adults. In this study, we investigated if current inputs to the river are capable of causing similar effects in the Miramichi River. From 1999-2001, we conducted sampling programs on the river and at major industrial and municipal sources. Concentrations of 4-nonylphenol in riverine samples are insufficient to implicate alkylphenols as a current problem. Levels found in the effluent samples were also below the estimated no-effects value (ENEV) for NP, NP1EO, NP2EO, NP1EC, NP2EC, OP1EC and OP2EC when a dilution factor of 10:1 is taken into consideration. Results of analysis for numerous chlorophenols, trace metals and PAHs were also completed. Only Al, Cr and Cu concentrations in water were consistently higher than Environment Canada guidelines. In sediments, values above Canadian Sediment Quality Guidelines were consistently found for As, Cr and Cu. Yeast screen assays indicated that the pulp mill effluent, municipal wastewater effluent and some river water samples were estrogenic. Androgenicity was also evident in effluents and some river water samples.

Effect of toxaphene on the liver of female yellowtail flounder (*Limanda ferruginea* **Storer).** G.E. Fåhræus-Van Ree and H.A. Baikie. Department of Biology, Memorial University of Newfoundland, St. John,s, NF.

Toxaphene, a synthetically-developed and once extensively-used organohalide pesticide, has been shown to be a potential endocrine disrupter in numerous organisms. This study investigated the effect of in vivo exposure to Toxaphene upon the liver of laboratory-bred juvenile female yellowtail flounder, *Limanda ferruginea*, (12.5-18 cm standard length, 39.8-100 g initial weight), sampled in early June. Fish were untreated (initial control) or exposed to Ringer's solution (blank control), corn oil and acetone (vehicle control), or Toxaphene (at concentrations of 0.2 or 2.0 μg/fish) by means of a one time, weight-dependent intraperitoneal injection. The injected fish were analyzed after either four or eight weeks. Liver tissues were examined histologically (structure, reticulin fiber network), histochemically (total and neutral lipids, glycogen, hemosiderin) and immunocytochemically (vitellogenin) and then analyzed by image analysis. Toxaphene negatively affected the structural integrity of the liver, and depending on the exposure time it decreased the relative amount of hemosiderin and increased that of total and neutral lipids. The relative amounts of glycogen and vitellogenin within the hepatocytes appeared not to be affected by this pesticide. Thus, Toxaphene can alter the structure of and lipid metabolism in the liver of yellowtail flounder, which may have consequences for the physiological processes involving the liver, such as storage of energy reserves, reproduction, and detoxification of xenobiotics.

An assessment of ambient water estrogenicity at sites along the St. Clair River. J.P. Sherry[1], C. Tinson[1], K. Cooper[1], M.E. McMaster[1], S.B. Brown, J.L. Parrott[1], T. Moran[2], T. Kierstead[2] and S. Munro[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Pollutech

EnviroQuatics, Point Edward ON; and [3]Sarnia Lambton Environmental Association, Sarnia, ON.
A large proportion of Ontario,s petroleum refining and petrochemical manufacturing base is located along the St. Clair River. The St. Clair River links Lake Huron to Lake St. Clair. Juvenile rainbow trout (*Oncorhynchus mykiss*) were caged at multiple sites along the St. Clair River in the autumn of 2002. The selected sites ranged from an upstream non-impacted site, through the zones of influence of a sewage treatment plant, several refineries and petrochemical plants, to a downstream reference site. There were 15 fish per cage and the exposures were for 21 d. Vitellogenin (Vg) in the plasma of the exposed fish was used as a biomarker of exposure to environmental estrogens. EROD activity in the livers of the exposed fish was used as a biomarker of exposure to planar aromatic hydrocarbons with the ability to bind to the Ah receptor.

'Fishy' business (agriculture)/Histoire d'eaux sales (agriculture)

Session Chairs/Présidents: E. Topp and/et W.R. Ernst

Evaluation of hormonal activities in municipal biosolids and manures. A. Lorenzen and E. Topp. Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON.
The potential exists for natural hormones or synthetic chemicals present in animal manures or municipal biosolids used as fertilizers in current agricultural practices to transfer to adjacent aquatic environments and alter endocrine function in exposed wildlife or humans. Three recombinant yeast assays and a mammalian cell line (BG1Luc4E2) were used to screen crude organic extracts of manures and biosolids for hormone receptor binding activities. To date, o 19 Ontario biosolid extracts tested with the recombinant yeast bioassays, 37% had detectable estrogen receptor binding activities, 53% had detectable androgen receptor binding activities and none had detectable progesterone receptor binding activities. When the same samples were tested with the BG1Luc4E2 cell line, 79% had detectable estrogen receptor binding activities and no significant anti-estrogenic activity was detected. Thirty-two liquid swine manure extracts were also tested with the recombinant yeast bioassays. Of these, 94% had detectable estrogen receptor binding activities and 25% had detectable androgen receptor binding activities. Experiments are underway to test extracts of different types of animal manure and to determine the persistence of the androgenic activity of testosterone and biosolid extracts in various types of soil. Results of these studies will provide important information that can be used to help implement farming practices that will have the least impact upon the environment and non-target species.

Fate of selected endocrine-disrupting substances in agricultural soils. E. Topp[1], A. Lorenzen[1], B.K. Burnison[2] and M.R. Servos[3]. [1]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; and [3]Canadian Water Network, University of Waterloo, Waterloo, ON.
Biosolids and animal wastes can contain natural hormones or synthetic chemicals that have the potential to disrupt endocrine function in wildlife should they move offsite. The persistence in soil of a number of estrogenic substances that could reach agricultural land via fertilization with organic amendments has been evaluated. 4-Nonylphenol, ethynylestradiol, estradiol and estrone are rapidly dissipated in soils under a range of conditions typical of a temperate growing season with half lives ranging from a few hours to a few days. The major pathway for 4-nonylphenol removal was mineralization, whereas for the steroidal estrogens it was formation of non-extractable soil-bound residues. Dissipation was much faster in the presence of oxygen, is microbially catalyzed, and in the

case of estradiol and estrone reduces concentrations to a detection limit of part per trillion. There was no detectable potential for matrix flow of estrogens through a sandy soil. We conclude that these chemicals are rapidly removed from aerated soils under temperate growing conditions, and application methods which minimize preferential flow or runoff of animal or human wastes should protect adjacent water from contamination with these chemicals.

Slimy sculpin (*Cottus cognatus*) **population responses in agricultural regions of northwestern New Brunswick.** M.A. Gray[1] and K.R. Munkittrick[2]. [1]Canadian Rivers Institute, University of New Brunswick, Fredricton, NB; and [2]Department of Biology, University of New Brunswick, St. John, NB. Over a period of three years, we monitored the population structure of slimy sculpin (*Cottus cognatus*) in an intensive cultivation region in northwestern New Brunswick. Aquatic stressors from row crop cultivation may include direct inputs such as pesticides, nutrients, and soil from fields following rainfall events, and indirect effects such as increased temperatures (e.g., associated with clearing of bank-side vegetation), habitat alteration, impacts on food sources, changes in local hydrology, and reduced dissolved oxygen. Rather than focus on particular stressors, an effects-based assessment of the fish in the system was conducted to determine whether there were observable and persistent responses in the agricultural region. The slimy sculpin, a small-bodied benthic fish, was a suitable candidate for sentinel monitoring due to a natural abundance throughout the system, limited mobility, lack of commercial pressures, and easily measured life history characteristics. We found that the local population structure at agricultural sites was modified, consisting of fewer (or no) young fish. Adult sculpin were larger, with smaller gonads, lower fecundity and reduced nest densities in waters receiving runoff from agricultural activities. A follow-up study suggested that altered temperature regimes play a significant role in determining fish size and density in agricultural streams at some sites. However, chemical exposures likely play a dominant role in acute lethality episodes and may be related to impacts on fecundity and organ size.

Relative risk ranking of pesticides used in Prince Edward Island. A.M. Dunn. Environment Canada, Atlantic Region, Dartmouth, NS.
Establishing priorities for regulatory and scientific assessments of pesticides is difficult given the large number of registered pesticide products and varied use patterns of each product. A relative risk ranking of agricultural pesticides was developed using a modified CHEMS model. According to the general paradigm of "Risk = Toxicity x Exposure," CHEMS tabulates a risk score by multiplying the sum of release-weighted toxicity endpoints (i.e., acute oral LD50, acute inhalation LC50, carcinogenicity rating, no observed adverse affect level, acute fish LC50, acute *Daphnia* EC50) by the sum of weighted exposure endpoints (i.e., water half-life, log BCF). Releases are integrated into the model by calculating media-specific release-weighting factors that are multiplied by their corresponding effect endpoints. While the model makes use of toxicity and exposure data, the resultant risk ranking does not represent a quantitative risk assessment. Rather, the results from CHEMS should be viewed qualitatively as its goal is to provide relative groupings of high, medium and low risk substances. Hazard and exposure endpoint data were collected for a subset of 31 active ingredients, representing 94% of PEI's 2001 pesticide sales. Model limitations included the use of pesticide sales data as a proxy for release and the reliance on modelled data for exposure parameters and determining environmental partitioning. In spite of its limitations, the CHEMS risk ranking scheme provides a useful tool for prioritizing pesticides of concern for future action.

An assessment of the effectiveness of 10 meter buffer zones in minimizing pesticide runoff from potato fields in Prince Edward Island. A. Denning[1], W.R. Ernst[1], M. Bernier[2], A. Cook[2], K.G.

Doe[2], P.M. Jackman[2] and G. Julien[2]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NB; and [2]Environment Canada, Environmental Conservation Branch, Moncton, NB.
In an effort to prevent aquatic impacts such as fish kills, 10 metre riparian buffers are now required of farmers in Prince Edward Island. In order to quantify the reduction in pesticide transport by those measures, samples were collected after large rainfall events at the edge of fields as well as at distances of 10-30 m in the buffer, and analyzed for un-ionized ammonia and pesticide (endosulfan, chlorothalonil, imidacloprid, azinphos-methyl and phorate) content in aqueous and particle phases. Toxicity to *Daphnia magna* of samples was also determined. There were substantial numbers of samples which exceeded CCME criteria for various pesticides, or published *Daphnia* toxicity concentrations at the edge of field, however only chlorothalonil (22%) and un-ionized ammonia (25%) exceeded the CCME criteria at 10 m. No contaminant concentrations exceeded guidelines at 30 m. Although the buffers were generally effective in reducing pesticide content of water samples, they were not consistently effective in reducing toxicity. Up to 40% of the samples obtained produced toxic effects at 30 m. Significant correlations were obtained with chlorothalonil and un-ionized ammonia concentrations and *Daphnia* toxicity.

Assessment endpoints for ecological risk assessments of pesticides: a scientific perspective. G. Kaminski, P. Delorme, C. Kriz, V. Hodge, H. Mulye, R. Sebastien, C. Hart, P. Takacs, D. François and T. MacQuarrie. Health Canada, Pest Management Regulatory Agency, Ottawa, ON.
Assessment endpoints are expressions of actual environmental values that we wish to protect. As part of their decision making process, risk managers use assessment endpoints as a link between the risk assessment and identified protection goals. The Environmental Assessment Division of the Pest Management Regulatory Agency sponsored a workshop to identify and characterize, from a scientific perspective, the ecological assessment endpoints that should be considered in the environmental assessment and risk management decisions for the registration of pesticides in Canada. Scientists from government, academia, NGO and industry were invited to participate. Discussions were organised along the taxonomic groups (plants, terrestrial invertebrates, aquatic invertebrates, fish, amphibians, birds, and mammals). Initial sessions examined the applicability of generic assessment endpoints to the different levels of biological organization (i.e., individuals, population, community and ecosystem). Subsequent sessions refined the selection of assessment endpoints by considering temporal, spatial and biological factors as well as agricultural factors (i.e., use patterns). The generic and refined assessment endpoints for each taxonomic group and their rationales will be presented. The outcome of the workshop will provide a scientific perspective for risk managers on environmental protection goals and provide risk assessors with clearer guidance on assessment endpoints for ecological risk assessment of pesticides.

"Fishy" business (aquaculture)/Historie d'eaux sales (aquaculture)

Session Co-chairs/Présidents: K. Haya and/et M. Ikonomou

Chemicals in finfish aquaculture in Canada: a review of current practices and possible environmental effects. L.E. Burridge. Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB.
Concerns have been raised within the scientific community for a number of years about the environmental consequences of chemical use in aquaculture. This review is a summary of potential sources of chemical contamination, chemicals that may be involved, and knowledge about environmental effects of these compounds. Food additives, anesthetics, drugs, pesticides, persistent

organic pollutants, metals and plastics are discussed in the context of the Canadian aquaculture industry. Generic gaps in our current state of knowledge regarding environmental effects are identified. The persistence of these compounds in sediment and biota, in most cases, is unknown. Toxicity data are limited to lethality tests conducted over short time frames. More work is required to determine chronic lethal and sublethal effects and the effects of realistic exposures of these compounds on indigenous species. While there are lab-derived data on many compounds, there is almost no information on effects in the field. Field studies that investigate short-term and long-term responses to chemical application, as well the natural variability in local populations and changes in biodiversity (and other indicators of environmental health) are needed. Toxicity testing relies on single species and single compound testing in the lab. There is a lack of data regarding the cumulative effect(s) of exposure to chemicals, and the concentration and fate of chemicals of aquaculture origin.

Impacts of freshwater aquaculture on the Canadian environment: towards science-based tools for sustainable management. S.L. Walker[1], S.S Dixit[2], D. Anderson[1], P.Y. Caux[3], P.A. Chambers[4], M.C. Chambers[4], L.A. Howes[5] and L. Kingsley[5]. [1]Environment Canada, National Environmental Effects Monitoring Office, Gatineau, QC; [2]Environment Canada, Water Policy and Coordination Directorate, Gatineau, QC; [3]Environment Canada, Conservation Program Integration, Gatineau, QC; [4]Environment Canada, National Water Research Institute, Burlington, ON; and [5]Environment Canada, Canadian Wildlife Service, Gatineau, QC.

Commercial aquaculture in Canada has rapidly expanded in the last 20 years. Aquaculture operations vary greatly across Canada. Some land-based facilities use recirculation and waste water treatment, thereby reducing inputs of nutrients and other chemicals. However, net cage operations generally disperse waste directly into public waters. Types of waste produced from feeding fish include solid (feces, uneaten feed, and organic matter) and soluble (dissolved phosphorus, ammonia, dissolved organic carbon, and lipids) material. As well, there are inputs of drugs, disinfectants and other chemicals as part of the ongoing operation and maintenance of the aquaculture operations. Environmental impacts of aquaculture depend on site characteristics, type, size and practices of the operation and the nature of the wastes released to the environment. The most common impact of aquaculture is nutrient enrichment. Other changes to aquatic ecosystems can include deterioration of water quality, changes in physical and chemical characteristics of the sediment, shifts in algal and invertebrate communities, increase of birds around net cages, and increased interactions, disease transmission, and competition between farmed and wild fish. A review of the existing environmental monitoring programs indicates a lack of consistency across Canada. Similarly, Canadian Environmental Quality Guidelines are lacking for many chemicals used in aquaculture. This presentation will review some of the work that is ongoing and proposed by Environment Canada to help address environmental issues pertaining to freshwater aquaculture.

Monitoring of therapeutants and phycotoxins in kelp (*Laminaria saccharina*) **and mussel** (*Mytilus edulis*) **cultured in proximity to salmon** (*Salmo salar*) **in an integrated system.** D.H. Sephton[1], K. Haya[1], J.L. Martin[1], G. Boyer[2] and T. Chopin[3]. [1]Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB; [2]College of Environmental Science and Forestry, State University of New York, Syracuse, NY; and [3]Department of Biology, University of New Brunswick, St. John, NB.

An AquaNet project on the economical, environmental and social feasibility of integrated aquaculture for salmon (*Salmo salar*), kelp (*Laminaria saccharina*) and blue mussel (*Mytilus edulis*) is in progress. This presentation is on the safety of the co-cultured kelps and mussels for human consumption. Concern is with potential transfer and accumulation of therapeutants used in treatment of diseases in cultured salmon and of phycotoxins produced by harmful algae. Kelps and mussels growing adjacent to salmon cages, in Bocabec Bay, Bay of Fundy, Canada, were collected periodically since May 2001. Kelp and mussel tissues were analyzed for eight therapeutants and two phycotoxins, paralytic shellfish poisoning (PSP) toxins (produced by *Alexandrium fundyense* and causes PSP in humans), and domoic acid (produced by *Pseudo-nitzschia pseudodelicatissima* and causes amnesic shellfish poisoning in humans).

None of the therapeutants or domoic acid was detected in kelp and mussel tissues. *Alexandrium fundyense* cells were present in water samples from May-October in 2001 and 2002 but peak abundances up to 1500 cells/L occurred only in mid June to late July. PSP toxins content in mussels peaked in late June in both years, lagging peak abundance of *A. fundyense* by 3-8 d. PSP toxins in mussels exceeded regulatory levels (RL) of 80 μg saxitoxin equivalence/100 g wet weight from late May to early July in 2001 and in late June in 2002. Mussels fed cultured *A. fundyense* in the laboratory accumulated PSP toxins well above RL in 4 d, and had different PSP toxins profile compared to that in field mussels.

Evaluation of the flesh quality of market-size farmed and wild British Columbia salmon. M.G. Ikonomou[1], D. Higgs[2], R. Devlin[2], B. Sukura[3], J. Oakes[2], S. McKinley[3], S. Jones[4], C. Dubetz[1], S. Balfry[2], J. Smith[2] and N. Rowshandeli[2]. [1]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC; [2]Department of Fisheries and Oceans, West Vancouver Laboratory, West Vancouver, BC; [3]University of British Columbia ,Vancouver, BC; and [4]Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC.

Presently, there is no comprehensive and reliable published information on the variability of the flesh quality of market-size farmed (Atlantic, chinook and coho) and wild (chinook and coho of interest here) BC salmon. Accordingly, this study was funded by AquaNet and the BC Salmon Farmers Association to compare the flesh quality of each of the three species of farmed salmon to wild Pacific salmon (sized-matched to cultured counterparts where possible). Specifically, the study will compare the flesh quality of the different sources of salmon with respect to the following: (i) concentrations of classical persistent environmental contaminants, (ii) concentrations of antibiotics and antimicrobials, (iii) concentrations of Hg and trace metals, (iv) concentrations of proximate constituents, (v) concentrations of fatty acids, (vi) concentrations of carotenoid pigments, (vii) sensory attributes, (viii) visual and instrumental characterization of flesh colour, and (ix) prevalence of the parasite *Kudoa*. Measurements of stress hormones and reproductive hormones and various haematological parameters in the blood will also be performed and these data will be related to sensory attribute data or will be used for assessing the reproductive and health status of the fish. The scope and implementation of the study will be presented.

Toxic effects of jellyfish envenomation on fish in the northeast Atlantic. B.D. Johnston[1], H. Irvine[2] and P.R. Boyle[1]. [1]School of Biological Sciences, University of Aberdeen, United Kingdom; and [2]North Atlantic Fisheries College, Shetland, United Kingdom.

The effect of exposure to jellyfish venom was investigated in Atlantic Salmon, *Salmo salar*, and North Atlantic Cod, *Gadus morhua* in the laboratory and in cage-cultured fish from aquaculture farms around the British Isles as part of EUROGEL, a European Union funded project to determine the socio-economic impacts of jellyfish. A dried, powdered preparation of jellyfish tentacles containing nematocysts was used to expose isolated gill cells, hepatocytes, and red blood cells *in vitro*. Cell viability and metabolic function were measured. Cage-cultured fish exposed to a jellyfish invasion were also sampled on site. Blood was removed into syringes washed with Na+-EDTA. Red and white blood cell counts and hemoglobin concentration were taken. Plasma was analysed for cortisol, ACTH, glucose, lactate, LDH, plasma Na+ and Cl-, and histaminic complexes. Gill arches and skin samples were removed and analysed visually for signs of oedema and degeneration. Preliminary results of cytotoxicity, tissue degeneration, stress and histamine responses in relation to exposure of fish to jellyfish venom or jellyfish invasion will be reported.

Potential endocrine disruption and mussel leukemia from finfish aquaculture. M.B. Engel[1], W.R. Ernst[1], S.D. St. Jean[2], F. Gagné[3] and B.K. Burnison[2]. [1]Environment Canada, Atlantic Region, Dartmouth, NS; [2]Environment Canada, National Water Research Institute, Burlington, ON; and [3]Environnement Canada, Centre Saint Laurent, Montréal, Qc.

In order to investigate the potential for adverse environmental effects from finfish aquaculture, blue mussels (*Mytilus edulis*) and sediments were collected near salmon aquaculture sites and analyzed for endocrine disruption endpoints as well as the incidence mussel leukemia. Collections were also made at municipal waste water sites, near industrial sites (pulp mill, fish processing plant), and control areas in the lower Bay of Fundy in November and December 2002. There was a significant difference in mussel condition between sites, with some of the highest indices found at aquaculture sites ($p=<0.001$). While leukemia rates were within background levels in Nova Scotia, there were elevated levels of leukemic cells near areas of anthropogenic activity in New Brunswick. Estrogenicity in sediments as measured by % estradiol activity using the yeast estrogen screen (YES) assay was low (generally less than 15% and often less than 5%) for both reference and treatment sites in water-extracted sediments. Analysis of vitellin-like proteins in soft mussel tissue revealed significant differences in Vn levels between sites. Both leukemia and the incidence of endocrine disruption are complex issues. The discovery of significant differences between sites warrants further investigation.

The effects of Salmosan® (Azamethiphos) on larval sea urchins and larval and juvenile scallops. L.E. Burridge, K. Haya and S.M.C. Robinson. Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB.

Salmosan®, active ingredient azamethiphos, is one of several pesticide and drug products available to salmon farmers to combat infestations of ectoparasitic copepods (sea lice). Salmon farmers administer the compound to caged salmon in a bath treatment of 100 µg/L (as azamethiphos) for up to 1 h. Use of pesticide formulations and the post-treatment direct release of the treated water into the marine environment has raised concerns regarding potential effects on indigenous species. Of particular concern is the effect on commercially important species. Twenty-four hour lethality tests were conducted with larval sea urchins (*Strongylocentrotus droebachiensis*) and with larval and juvenile scallops (*Placopecten magellanicus*). Sub-samples of cultures of larval urchins and scallops of known density were exposed to a range of azamethiphos concentration for 24 h. Juvenile scallop of three different sizes (fine, medium, and coarse) were similarly exposed in azamethiphos-treated seawater. Survival was assessed at 3, 6, 12 and 24 h by counting the number of live and dead individuals at each concentration. Exposure of these invertebrates to concentrations up to 300 µg/L (3 times the recommended treatment concentration) resulted in no treatment-related mortality. These data indicate that the use of Salmosan® poses no lethal threat to these species.

Persistent environmental contaminants measured in the sediments near salmon aquaculture sites. M.G. Ikonomou[1], J. Hellou[2], K. Haya[3] and P. Sather[1]. [1]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC; [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; and [3]Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB.

As aquaculture is expanding continuously, concerns over the potential impact of fish-farms on the coastal environment are increasing. One area of special concern relates to the level of trace organic compounds remaining in the sediments beneath and around fish farms. In this study sediments from four types of fish farm environments (hypoxic, normoxic, anoxic and a remediation site) were examined. Sampling covered a distance ranging from under, i.e., 0-100 m away from the fish farm pens. Samples were analysed for a variety of organic contaminants including polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polyaromatic hydrocarbons (PAHs), and classical organochlorine pesticides. For example, the PCBs show a congener distribution similar to a mixture of Aroclors of 1:1:1 (Aroclors 1242:1254:1260); the PBDEs show a dominant congener (209 present as 42-84% of the total) with several other major congeners (47, 99, 183 and 207); and the PCDD/Fs are dominated by OCDD (~80%) followed by 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDF, and OCDF. As well, combustion PAHs are more abundant than those derived from fossil fuels (sum of alkylated PAH is lower than parental PAH). Total concentrations of each type of contaminant tend either to rise and then fall or to remain roughly similar over all distances depending on the site.

Antibiotic resistant aerobic bacteria and oxytetracycline in sediments collected near salmon aquaculture sites in the Fundy Isles region, New Brunswick, Canada. K. Haya[1], B.T. Hargrave[2], K.G. MacKiegan[1], L.I. Doucette[2], S.M. Armstrong[3] and F.S. Friars[3]. [1]Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB; [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; and [3]Department of Biology, Dalhousie University, Halifax, NS.

Salmon feed medicated with antibiotics is used to treat bacterial infections of cultured salmon. In New Brunswick the antibiotic of choice is oxytetracyline (OTC). OTC may accumulate in sediments from sedimentation of excess food pellets and fish wastes near farm sites and may lead to the selection, development and growth of oxytetracycline resistant populations of bacteria. Resistance to OTC was determined in aerobic bacteria cultured from surface sediments collected from under, near (up to 100 m) and distant from salmon aquaculture sites in the Fundy Isles Region of New Brunswick. Sediment collected within 100 m of active salmon aquaculture sites in late summer of 2000 indicated the presence of aerobic bacteria with OTC resistance above a minimum inhibitory concentration (MIC) > 25 μg OTC/ml. Controls consisting of a culture of *Areomonas salmonicida* and bacteria cultured from sediments collected from reference areas where salmon aquaculture does not occur (St. Ann's Harbour) showed no resistance to OTC (MIC < 10 μg OTC/ml). Sediment extracts were analyzed for OTC by LC-MS. OTC was found in sediment from 6 of 8 salmon aquaculture sites sampled in 2000 at concentrations ranging from 5-450 μg/kg. In some cases the concentration of OTC increased as the distance from the salmon cages increased, suggesting that the OTC treatments had occurred some time prior to the sampling period. Results from sediment collected in May and September of 2002 were comparable.

Best student paper awards/Prix pour les meilleurs exposés par des étudiants

Best Platform Paper Award
Nil Basu, McGill University
Muscarinic receptor: a novel biomarker of mercury neurotoxicity in wildlife

Best Poster Paper Award
Vivian Dayeh, University of Waterloo
Developing rapid toxicity tests with the ciliated protozoan, *Tetrahymena thermophila*, that utilize microwell filter plates

Other platform paper awards:
2nd. Adrienne Bartlett, University of Waterloo
3rd. Stephanie Hawkins, Queen's University; and Warren Norwood, University of Waterloo
Honourable Mentions: Michelle Bowerman, Queen's University; Denis Brion, Université du Québec à Rimouski; John Dungavell, Queen's University; Ève Dussault, University of Guelph; Kimberly Hruska, University of Saskatchewan; Caroline Mimeault, University of Ottawa; Devi Ramachendran, Queen's University; Ahmed Siah, Université du Québec à Rimouski; Christian Wilson, University of Guelph; and Amanda Woodhouse, University of Ottawa.

Other poster paper awards:
2nd. Allison Squires, University of Saskatchewan
3rd. Kimberly Hruska, University of Saskatchewan; and Warren Norwood, University of Waterloo
Honourable Mentions: Lyndon Barr, University of Windsor; Winnie Cheng, Simon Fraser University; Maria Colavecchia, Queen's University; Maxine Croteau, University of Ottawa; Barbara Perez, University of Ottawa; Carrie Rickwood, University of Saskatchewan; and Carla Wytrykush, University of Windsor.

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List of authors/List des auteurs

Page number in bold indicate first author

Acreman, J.C.: **12**
Akhtar, P.: 46,**47**
Ali Khan, A.: 111
Allen, M.S.: 23
Amiard, J.-C.: 54,118
Amiard-Triquet, C.: 38
Ampleman, G.: 93
Anderson, D.: 138
Andrews, C.: 116
Appleton, M.: 76
Aravindakshan, J.: 131
Armstrong, S.M.: 141
Aston, P.: 113
Atkinson, A.J.: 74,81
Au, A.: 18
Babu, T.S.: 15
Bacigalupo, C.: 30
Backus, S.: 16
Baikie, H.A.: 134
Balch, G.C.:**128**,128, **129**,**133**
Baldisserotto, B.: 37
Balfry, S.: 139
Baron, C.L.: 25
Barr, L.: **53**
Bartlett, A.J.: **13**
Basu, N.: **31**
Batchelor, S.P.: 13
Bauder, M.: 15
Becker-van Slooten,K.: 91
Belknap, A.: 132
Bennie, D.T.: **134**
Bernier, M.: 136
Berthelot, Y.: 93
Bielak, A.T.: **111**
Birkholz, D.A.: **73**
Bishay, F.: 23
Bisson, M.: 110
Blaise, C.: 61,114,127
Blanchfield, P.J.: 132
Bols, N.C.: 37,48,52
Bombardier, M.: 109
Borgmann. U.: 13,15, 29,34
Bortolotti. G.R.: 45
Bowerman, M.E.: **54**
Bowman, M.F.: **62**
Boyer, G.: 138
Boyko, C.: 132
Boyle, P.R.: 139
Braekevelt, E.: 48
Brain, R.: 117,127
Brigham, S.: 26
Bright, D.A.: 91
Brion, D.: **94**
Brockbank, R.: 73
Brown, K.: **1**
Brown, M.: 113
Brown, R.S.: 46,47,74,**113**
Brown, S.B.: 45,134,134
Buckman, A.: **45**
Budakowski, W.: 48
Buday, C.: **110**
Burnison, B.K.: 129, 134,140
Burridge, L.E.: **137**,**140**
Bursian, S.: 49
Butler, B.J.: 83
Butler, M.: 108
Cairns, G.: 113
Cameron, M.: 34,52
Campbell, P.G.C.: 35,36
Caron, D.: 18
Caux, P.Y.: 138
Chakrabarti, C.L.: **33**
Chambers, M.C.: 138
Chambers, P.A.: 138
Chan, H.M.: 31
Chapman, P.M.: **31**
Chassé, R.: 108
Chaudry, O.: 109
Cheburkin, A.: 32
Cheng, W.W.L.: **78**
Chong-kit, R.: **76**,76
Chopin, T.: 138
Ciborowski, J.J.H.:**34**,53, **72**,82
Clarke, J.D.: **115**
Clarke, L.M.: 18,**74**
Cloutier, I.: 20
Colavecchia, M.V.: **16**,**18**
Collins, A.: 76
Cook, A.: 136
Cook, R.: **20**
Cooper, N.: 53
Cooper, K.: 134
Cossu-Leguille, C.: 35
Couillard, C.M.: **16**
Courchesne, F.: **32**
Courtenay, S.C.: 21,23
Couture, P.: 35,**36**
Craig, G.R.: **115**
Croft, M.V.: 52,74
Croisetiere, L.: **34**
Cross, T.: 45
Croteau, M.C.: **77**
Crumb, J.T.: **91**,92
Culp, J.M.: 22,22,72
Cyr, M.: 110
Cyr, D.G.: **131**
Davies, T.: 26
Dayeh, V.R.: **48**,**52**
De Serres,J.-P.: **1**
DeBlois, E.: 13
Dehn, P.F.: 47
Delaronde, J.: 30
Delorme, P.: 137
Denning, A.: **136**
Devlin, R.: 139
Diamond, M.: 74
Dimacacos, C.: 133
Diringer, E.: **47**
Dixit, S.S.: 72,138
Dixon, D.G.:13,15,16, 29,34,52,52,83,93
Dodard, S.: 93,**93**
Doe, K.G. : 136
Doetzel, L.M.: **30**
Doucette, L.I.: 141
Doyle, P.J.: 18,29
Dubé, M.G: 22,22,25,27
Dubetz, C.: 139
Dungavell, J.: **45**
Dunn, A.M.: **136**

Durou, C.: 38
Dussault, É.B.: **129**
Eales, J.G.: 48
Eberius, M.F.: **94**
Ellison, D.: **115**
Engel, M.B.: **140**
Ennis, J.: 45
Ernst, W.R.: **136**,140
Evans, D.: 31
Evans, M.S.: 30
Evans, R.E.: 25,132
FåhræusVanRee,G.E.: **134**
Fairchild, W.L.: 134
Falicki, S.: 74
Fancey, L.L.: 116
Farrell, A.P.: 78
Farwell, A.J.: 16,**52**,**83**
Fasfous, I.I.: 33
Feisthauer, N.C.: 91, 107,107
Ferguson, G.M.: 30,108
Findlay, D.L.: 132
Findlay, R.: **10**
Firth, B.K.: 132
Fisk, A.T.: 45
Foote, L.: 53
Foran, C.M.: 129
Fouchécourt, M.: 110
Fournier, M.: 133
François, D.: 137
French, B.: 116
Friars, F.S.: 141
Gagné, F.: 61,76,114,140
Gagnon, C.: **114**
Gallant, N.: 77
Gallon, C.: **32**
Gamberg, M.: 31
Gardner, M.: 75
Gewurtz, S.: 74
Gibson, J.: 77
Giddings, M.: 112
Giguère, A.: **35**
Gilbertson, M.: 107,107
Gilmour, C.: 113
Gilron, G.: **30**,**73**
Gobeil, C.: 32
Gong, P.: 93
Goudey, J.S.: 73
Goulet, R.R.: **29**,31
Gratas, C.: 54
Gray, M.A.: 136
Greenberg, B.M.: **15**
Gregorich, E.: 109
Grosell, M.: 37
Hamilton, A.L.: **10**
Hammond, G.: 74,81
Hansen, P.D.: 130
Hare, L.: **28**,29,34, 35,35,36
Hargrave, B.T.: 141
Hart, C.: 137
Hawari, J.: 93,93
Hawkins S.: **17**,**17**
Hawson, C.: 32
Haya, K.: 138,140,140,**141**
Hedley, K.: **19**,22,72
Hedley, C.: 17
Helbing, C.C.: 131
Hellou, J.: 49,140
Hendershot, W.: **33**
Hewitt, L.M.: 20,**22**,22,**132**
Higgs, D.: 139
Hilderman, M.: 112
Hock, B.: 130
Hodge, V.: 137
Hodson, P.V.: **15**,16,17,18, 18,45,46,47,54,74
Hollebone, B.: 18
Hollenhorst, T.: 72
Hontela. A.: **44**
Hooey, T.: 130
Host, G.E.: 72
Hostetler, B.: 113
Howes, L.A.: 138
Hruska, K.A.: **25**,**27**
Huang, X.D.: 15
Hull, R.N.: 30,108
Huras, C.A.: **26**
Ibrahim, P.: 23
Ikonomou, M.G.: **139**,**140**
Irvine, H.: 139
Jackman, P.M.: 136
Janoscik, T.: 132
Jefferson, C.: 113
Ji, L.: 131
Jiapizian, P.B.: **110**,112
Johnson, D.J.: 117,127
Johnson, L.B.: 72
Johnston, B.D.: **46**,**139**
Jones, S.: 139
Julien, G.: 136
Kaminski, G.: **137**
Kamunde, C.: 31,37
Karalis, E.: **81**
Keating, J.: 30
Kennedy, C.J.: 46
Khan, C.W.: 17,**18**,54
Khan, H.: 111,**112**
Kidd, K.A.: 74,**132**
Kierstead, T.:**75**,**134**
Kilgour, B.W.: **72**
Kim, K.K.J.: 23
King, M.M.: 26
Kingsley, L.: 138
Kiparissis, Y.: 46
Klenavic, K.: 31
Kollar, S. : 25
Kovacs, T.G.: 20
Kraemer, L.: 36
Kriz, C.: 137
Kuperman, R.: 91
Lacroix, A.: 44
Lafortune, A.: **108**
Lampi, M.: 15
Lander, L.S.: **82**
Langlois, C.: 24
Lapointe, M.J.: 81
Larose, C.B.: **115**
Lau, B.: 48
Lawuyi, S.: 74
Lean, D.R.S.: **1**,77,77
Lee, E.: 113
Lee, H.-B.: 129
Lee, K.: 16,147
Lee, L.E.J.: 16,**23**,37,48,52
Legare, B.: 16
Legrand, P.: 32
Leonard, J.D.: **49**
Ley, A.: 113
Liber, K.: 109
Lister, A.: 16
Lo, A.: 1
Lock, A.: 113
Lockhart, W.L.: 30
Lorenzen, A.: **135**,135
Low, G.: 30

Lowell, R.B.: **22**
Lynn, D.: 48
Mabury, S.A.: 130
Macaulay, T.: 112
MacDonald, D.: 33
MacKiegan, K.G.: 141
Mackinnon, M.D.: 83
MacLatchy, D.L.: 22,22
MacQuarrie, T.: 137
Maguire, R.J.: 13
Marcotte, E.J.: 113
Martel, L.: 108
Martel, P.H.: **20**
Martin, G.: 1
Martin, J.L.: 138
Martin, P.: 49
Matteau, I.: 24
Mautner, S.: 109
Mayne, G.: 49
McCann, J.H.: **93**,107
McDonald, G.D.: 35,37
McDowell, S.: 133
McGeer, J.C.: 26
McKinley, E.: 133
McKinley, S.: 139
McLeay, D.: 91
McMaster, M.E.: **20,132**, 132,134
Metcalfe, C.D.: 117,117, 128,128,1329,133
Middelraad, I.: 116
Mihaich, E.: 129
Mikhail, M.: **24**
Miller, J.A.: 76,91
Mills, K.H.: 132
Mimeault, C.: **117**
Misra, R.K.: **84**
Misra, S.: 84
Moon, T.W.: 117,117,128
Moran, T.: 75,134
Morin, K.: 110
Mouneyrac, C.: **38**
Mueller, M.: 76
Muir, D.C.G.: 30,82
Mulye, H.: 137
Munkittrick, K.R.: 22,72, 132,136
Munro, S.: 75,134
Munson, B.A.: 115
Murimboh, J.: 33
Nadeau, J.C.: 26,32
Nero, V.: **16**
Nichols, J.: 37
Niimi, A.J.: **116**
Niyogi, S.: **37**
Noble, C.: 15
Norwood, W.P.: **15,29**,34
Novak, L.: 91
Nowierski, M.: 34
Nykamp, J.: 15
O'Toole, S.: 128
Oakes, J.: 139
Okonski, A.: 18
Orr, H.L 15
Orr, P.: **27**
Orvoine, J.: **35**
Osmond, D.: **24**
Ovaska, K.: 131
Paine, M.D.: **13,27**
Palace, V.P.: **25**,25,**48**, 49,132
Paquet, L.: 93
Paquet, V.: 131
Park, B.J.: 132
Park, L.: 116
Parker, W.R.: 21
Parrott, J.L.: 16,18, 20,22,134
Pastershank, G.: 72
Paterson, M.J.: 132
Paterson, R.: 111
Pauli, B.D.: 77
Payne, J.F.: **116**
Pearson, D.A.: 113
Pearson, F.: 24
Pedlar, R.: 27
Pellerin, J.C.: 38,**54**,61,118
Pelletier, É.: 94,118
Perez, B.: **77**
Peterson, B.: 129
Phommavong, T.: **112**
Pickard, J.: **26**
Playle, R.C.: **14,37**
Plohman, J.: 48
Podemski, C.L.: 132
Poirier, D.G.: 76,**76**
Poland, J.: 113
Portt, C.: 72
Power, F.: 13
Princz, J.I.: **92**
Proczkowski, J.: 73
Providenti, M.: **109**
Pryce, A.: 132
Pyle, G.: 36
Quereshi, P.: 15
Quinn, B.M.: **127**
Rainbow, P.: 38
Rajotte, J.: 36
Ralitsch, M.: 73
Ramachandran, S.: **18,47**
Rasmussen, J.B.: 35
Ray, J.: 113
Reiber, K.: 133
Reid, R.A.: 62
Reitsma, T.: 117
Renoux, A.: 108,**108,110**
Rhodes. S.M.: 52
Ribey, S.: 22
Richard, J.: 32
Richards, C.: 72
Richer, É.: 110
Rickwood, C.J.: **22**
Roach, P.: 74
Robidoux, P.Y.: **93**
Robinson, P.: 18,**53**
Robinson, S.M.C.: 140
Ross, G.: **25**
Ross, H.: 25
Rossiter, J.: 25
Rowshandeli, N.: 139
Roy, J.L.: 93
Ruby, S.M.: **133**
Russel, C.: 27
Ryan, M.J.: **74**
Safruk, A.: 73
Saint-Louis, R.: 118
Salazar, M.H.: **14,21**, **28**,114
Salazar, S.M.: 21,28
Salki, A.G.: 132
Sanderson, H.: 117,127,130
Sarrazin, M.: 93,93
Sather, P.: 140
Sauvé, S.: 32,33
Savard, K.: 93
Schetagne, R.: **30**

Scheuhammer, A.M.: 31
Schnabel, S.: 18
Schneider, K.D.: **92**
Schroeder, J.: 34,76,76,83
Schryer, R.: 132
Schudoma, D.: **76,83**
Schuldt, J.A.: 72
Schwartz, M.L.: 26
Scroggins, R.P.: 76,**91**,91, 92,92,109
Sebastian, M.: **45**
Sebastien, R.: 137
Séguin, V.: 32
Sekela, M.: 130
Sene, A.: 18
Sephton, D.H.: **138**
Servos, M.R.: 129
Sheppard, S.: **106**
Sherry, J.P.: 129,**130**,134
Siah, A.:**118**
Sibley, P.: 92,130
Siciliano, S.: 91
Simmons, D.B.: **128**
Singer, T.: 81
Smith, J.: 139
Smith, M.: 109
Smith, S.: 14,37
Smits, J.E.G.: 14,37
Solomon, K.R.: 45,117, 127,**130**
Somers, K.M.: 62
Sotornik, D.: 37,48
Spiers, G.A.: **32,113**
Squires, A.J.: **109**
St. Jean, S.D.: **21,23**, 140
St. Pierre, S.: 16
Stephenson, G.L.: 91,91, 92,93,106,**107**,107
Stern, G.A. 30,74
Sukura, B.: 139
Sullivan, C.A.: 134
Sunahara, G.I.: 93,93
Sylvestre, S.: 130
Tabash, S.P.: 15
Taillon, K.: 33
Takacs, P.: 137
Tarnowski, H.E.: 54
Taylor, L.A.: 115
Tessier, A.: 28,32,34,35
Tetreault, G.R.: 132
Thiboutot, S.: 93
Thomas, V.G.: 116
Tinson, C.: 134
Tittlemeir, S.: 48
Tomy, G.: 48
Topp, E.: 135,**135**
Tost, J.: 27
Trépanier, J.: 108,108,110
Trudeau, V.L.: 77,77,**117**, 117,128
Trudel, K.: 73
Tuominen, T.: 130
Turcotte, D.: **46**
Turcotte, P.: 114
Turmel, M.: 32
Van Aggelen, G.C.: 131
Van den Heuvel,M.R: 17, 17
Van Der Kraak, G.J.: 16,128,132
Van Pollelen, P.: 23
Veldhoen, N.: 131
Voss, R.: 20
Wagg, S.K.: 23
Walker, S.L.: **138**
Wallace, A.: 34
Wallis, P.: 110
Wang, F.: 31
Wang, Z.: 18
Watson, G.D.: 25,27,108
Watson-Leung, T.L.: 75,**83**
Wautier, K.L.: 25
Welnhofer, E.: 47
Werner, J.: 25
Whiteway, S.A.: 13,116
Wickstrom, G.: **34**,52
Willfang, S.B.: 23
Williams, L.: 25
Williams, U.: 13
Wilson, C.J.: 117,**127**
Wilson, P.A.: 128
Winter, A.: 14,37
Wood, C.M.: 37
Woodhouse, A.J.: **128**
Wren, C.D.: 108
Wytrykush, C.M.: 82
Xie, F.: 15
Yapici, T.: 33
Zhang, F.: 131

List of participants/Liste des participants

Last Name	First Name	Affiliation	Telephone	Email
Abernethy	Scott	Ontario Ministry of Environment	519-873-5118	scott.abernethy@ene.gov.on.ca
Acreman	Judy	University of Toronto	416-978-3641	jacreman@botany.utoronto.ca
Akhtar	Parveen	Queen's University	613-533-6000	akhtarp@chem.queensu.ca
Ali	Nardia	Environment Canada	416-739-5884	Nardia.Ali@ec.gc.ca
Andersen	Don	Environment Canada	819-953-7919	donald.andersen@ec.gc.ca
Andrews	Catherine	Dept. of Fisheries and Oceans	709-772-4396	andrewsc@dfo-mpo.gc.ca
Au	Alice	Environment Canada	819-994-3237	alice.au@ec.gc.ca
Baddaloo	Earle	Nunavut Research Institute	867-979-7202	ebaddaloo@nac.nu.ca
Baker	Mark	Environment Canada	519-823-9871	mbaker999@sympatico.ca
Balch	Gordon	Trent University	705-748-1011	gbalch@trentu.ca
Barr	Lyndon	University of Windsor	519-253-3000	barr5@uwindsor.ca
Bartlett	Adrienne	University of Waterloo	905-336-4405	adrienne.bartlett@ec.gc.ca
Basu	Nil	McGill University	514-398-7604	niladri.basu@mail.mcgill.ca
Beck	Andrew	Health Canada	613-952-8084	andrew_beck@hc-sc.gc.ca
Beers	Chris	Revelstoke	250-837-2124	chrisbeers@revelstoke.net
Berman	Rejean	Schering-plough Sante Animale	514-428-7487	rejean.berman@spcopr.com
Bernard	Donald	Environment Canada	604-664-4051	don.bernard@ec.gc.ca
Bielak	Alex	Environment Canada	905-336-4503	alex.bielak@ec.gc.ca
Bishay	Farida	Greater Vancouver Regional Dist.	604-451-6005	farida.bishay@gvrd.bc.ca
Blenkinsopp	Sandra	Environment Canada	780-951-8750	Sandra.Blenkinsopp@ec.gc.ca
Bombardier	Manon	Environment Canada	613-991-4062	manon.bombardier@ec.gc.ca
Borgmann	Uwe	Environment Canada	905-336-6280	uwe.borgmann@ec.gc.ca
Boucher	Nathalie	Lab-bell	819-539-8508	nboucher@lab-bell.com
Bowerman	Michelle	Queen's University	613-533-6000	bowermam@biology.queensu.ca
Bowman	Michelle	Trent University	705-766-1790	Michelle.Bowman@ene.gov.on.ca
Brion	Denis	ISMER	514-842-8756	denis_brion@hotmail.com
Brown	Karen	Environment Canada	819-997-2161	karen.brown@ec.gc.ca
Brown	Maureen	Environment Canada	780-951-8744	MaureenA.Brown@Ec.gc.ca
Brown	Scott	Environment Canada	905-336-6250	scott.brown@ec.gc.ca
Brown	Stephen	Queen's University	613-533-2655	browns@chem.queensu.ca
Brown	Terry	Bruce Power	519-361-2673	terry.brown@brucepower.com
Buchanan	Randy	Buchanan Environment	506-450-4463	buchenv@fundy.net
Buckman	Andrea	University of Guelph	905-336-4995	andrea.buckman@ec.gc.ca
Buday	Craig	Environment Canada	604-924-2514	craig.buday@ec.gc.ca
Burridge	Les	Dept. of Fisheries and Oceans	506-529-5903	burridgel@dfo-mpo.gc.ca
Byers	Tim	Byers Enviro-Studies	204-886-4642	byerses@escape.ca

Cameron	Marc	Keystone Environmental Ltd.	250-494-5310	marc.cameron@shaw.ca
Campbell	Peter	Université du Québec	418-654-2538	campbell@uquebec.ca
Casey	Richard	Government of Alberta	780-427-1583	Richard.Casey@gov.ab.ca
Caux	Pierre-Yves	Environment Canada	819-953-0602	pierre-yves.caux@ec.gc.ca
Chabot	Raymond	Environment Canada	514-283-0204	raymond.chabot@ec.gc.ca
Chakrabarti	Chuni	Carleton University	613-520-2600	chuni_chakrabarti@carleton.ca
Cheng	Winnie	Simon Fraser University	604-291-3977	wwcheng@sfu.ca
Chénier	Robert	Environment Canada	819-953-1680	robert.chenier@ec.gc.ca
Chong-kit	Richard	Ontario Ministry of Environment	416-235-5788	richard.chongkit@ene.gov.on.ca
Ciborowski	Jan	University of Windsor	519-253-3000	cibor@uwindsor.ca
Clarke	John	Environment Canada	902-426-6135	john.clarke@ec.gc.ca
Clarke	Lauren	Queen's University	613-533-6000	clarkel@biology.queensu.ca
Clarke	Trion	ASI Group Ltd.	905-641-0941	tclarke@asi-group.com
Colavecchia	Maria	Queen's University	905-336-4471	Maria.Colavecchia@cciw.ca
Constable	Miles	Environment Canada	780-951-8732	miles.constable@ec.gc.ca
Couillard	Catherine M.	Dept. of Fisheries and Oceans	418-775-0681	CouillardC@dfo-mpo.gc.ca
Couillard	Yves	Environment Canada	819-997-7588	yves.couillard@ec.gc.ca
Courchesne	François	Université de Montréal	514-343-8012	francois.courchesne@umontreal.ca
Couture	Patrice	Institut Nat. de la Recherche Sci.	418-654-3825	patrice_couture@inrs-ete.uquebec.ca
Craig	Gordon	G.R.Craig & Associates	905-859-3701	gordon@grcraig.com
Croisetiere	Louis	Institut Nat. de la Recherche Sci.	418-654-2595	louis_croisetiere@inrs-ete.uquebec.ca
Croteau	Maxine	University of Ottawa	819-569-9009	maxinecroteau@hotmail.com
Crumb	Jill	Stantec Consulting Ltd.	519-836-6050	jcrumb@stantec.com
Culp	Joseph	Environment Canada	506-458-7458	joseph.culp@ec.gc.ca
Cyr	Daniel	Inrs-institut Armand Frappier	514-630-8833	daniel.cyr@inrs-iaf.uquebec.ca
Czarnecki	Andrea	Indian Northern Affairs Canada	867-669-2509	czarneckia@inac.gc.ca
Davidson	Nicole	Environment Canada	819-997-3253	nicole.davidson@ec.gc.ca
Davies	Martin	Hatfield Consultants Ltd.	604-926-3261	mdavies@hatfieldgroup.com
Dayabhai	Dharmesh	Streampoint Solutions	416-743-7690	dharmesh@streampoint.biz
Dayeh	Vivian	University of Waterloo	519-885-1211	vrdayeh@sciborg.uwaterloo.ca
De Serres	Jean-philippe	Domtar, Inc.	514-848-5668	jean-philippe.d@domtar.com
Dehn	Paula	Canisius College	716-888-2555	dehn@canisius.edu
Dejong	Maryl	Environment Canada	905-336-6262	maryl.dejong@ec.gc.ca
Delage	Danaelle	Environment Canada	829-994-5823	danaelle.delage@ec.gc.ca
Delorme	Peter	Health Canada	613-736-3729	peter_delorme@hc-sc.gc.ca
Denning	Allison	Environment Canada	902-426-7780	Allison.Denning@ec.gc.ca
Desjardins	Sylvie	Environment Canada	819-997-1535	sylvie.desjardins@ec.gc.ca
Dick	Paul	Elanco	519-821-0277	p.dick@elanco.com
Dickson	Alison	Environment Canada	819-934-2371	alison.dickson@ec.gc.ca

Dixit	Sushil	Environment Canada	819-997-5880	sushil.dixit@ec.gc.ca
Dixon	D. George	University of Waterloo	519-888-4567	dgdixon@uwaterloo.ca
Dodard	Sabine	National Res. Council of Canada	514-496-2663	sabine.dodard@cnrc-nrc.gc.ca
Doetzel	Lyndsay	University of Saskatchewan	306-975-4132	lyndsay.doetzel@ec.gc.ca
Domey	Norma	Environment Canada	819-997-3204	norma.domey@ec.gc.ca
Doyle	Pat	Environment Canada	819-953-1590	patrick.doyle@ec.gc.ca
Doyon	Jean-françois	Association Minière Du Québec	418 657-2016	jfdoyon@amq-inc.com
Dufour	Éric	Bodycote Essais De Mat. Can.	418-871-8722	dufour.e@bodycote.ca
Dumaresq	Charles	Environment Canada	819-953-8459	charles.dumaresq@ec.gc.ca
Duncan	William	Teck Cominco Metals Ltd.	250-364-4336	bill.duncan@teckcominco.com
Dungavell	John	Queen's University	613-533-6000	9jad2@qlink.queensu.ca
Dunn	Allison	Environment Canada	902-426-5037	Allison.Dunn@ec.gc.ca
Dussault	Ève	University of Guelph	519-824-4120	edussaul@uoguelph.ca
Eberius	Matthias	Lemnatec, Wuerselen, Germany	49-2405412615	eberius@lemnatec.de
Elliott	Barbara	Environment Canada	819-953-9232	barbara.elliott@ec.gc.ca
Elliott	Garth	Environment Canada	780-435-7242	garth.elliott@ec.gc.ca
Elshayeb	Monalisa	University of Waterloo	416-993-0110	monalisaelshayeb@hotmail.com
Engel	Monika	Environment Canada	902-426-9405	monika.engel@ec.gc.ca
Evans	Robert	Dept. of Fisheries and Oceans	204-983-5006	EvansR@DFO-MPO.gc.ca
Fahraeus Van Re	Goverdina	Memorial Univ. of Newfoundland	709-737-7499	gvanree@mun.ca
Farara	Dennis	Stantec Consulting Ltd.	905-794-2325	DFarara@stantec.com
Farwell	Andrea	University of Waterloo	519-885-1211	afarwell@sciborg.uwaterloo.ca
Feisthauer	Natalie	Stantec Consulting Ltd.	519-836-6050	nfeisthauer@stantec.com
Ferone	Jenny-Marie	Environment Canada	780-951-8754	jenny.ferone@ec.gc.ca
Filion	Michel	Teck Cominco Metals Ltd.	604-687-1117	michel.filion@teckcominco.com
Findlay	Rick	Pollution Probe	613-237-8666	rfindlay@pollutionprobe.org
Firth	Barry	Weyerhaeuser	253-924-6946	barry.firth@weyerhaeuser.com
Flaborea	Dawne	City of Ottawa	613-831-8701	Dawne.Flaborea@ottawa.ca
Fletcher	Tim	Ontario Ministry of Environment	416-327-5002	tim.fletcher@ene.gov.on.ca
Forster-clegg	Lori	Environment Canada	780-951-8868	lori.forster-clegg@ec.gc.ca
Fraikin	Chris	Golder Associates	403-261-3021	cfraikin@golder.com
Gagné	François	Environment Canada	514-496-7105	francois.gagne@ec.gc.ca
Gagnon	Alexandra	Universite du Quebec a Montreal	450-625-3629	Gagnon_Alexandra@hotmail.com
Gagnon	Christian	Environment Canada	514-496-7096	christian.gagnon@ec.gc.ca
Gallant	Natacha	University of Ottawa	613-236-2885	ngallant@science.uottawa.ca
Gallon	Céline	INRS-ETE	418-654-2584	celine_gallon@inrs-ete.uquebec.ca
Gardiner	Elizabeth	Mining Association of Canada	613-233-9392	egardine@mining.ca
Gauthier	Joel	Environment Canada	819-994-6445	joel.gauthier@ec.gc.ca
Geadah	Marie-louise	Environment Canada	819-953-1129	marie-louise.geadah@ec.gc.ca

Gibson	Jennifer	University of Ottawa	613-562-8000	jgibs017@science.uottawa.ca
Giguère	Anik	Inrs-ete	418-522-6101	agiguere@mediom.qc.ca
Gilbertson	Mary-kate	C. Wren and Associates	519-766-1039	mgilbertson@cwren.com
Gilron	Guy	Cantox Environmental Inc.	905-814-7800	ggilron@yahoo.com
Gray	Colin	Environment Canada	604-664-4002	colin.gray@ec.gc.ca
Gray	Michelle	University of New Brunswick	506-447-3373	r55me@unb.ca
Greenberg	Bruce	University of Waterloo	519-888-4567	greenber@uwaterloo.ca
Guay	Isabelle	Quebec Ministry of Environment	418-521-3820	isabelle.guay@menv.gouv.qc.ca
Gutzman	Don	Environment Canada	613-994-0583	Don.Gutzman@ec.gc.ca
Hagen	Mike	Environment Canada	604-666-6544	mike.hagen@ec.gc.ca
Hall	Murray	Halltech Aquatic Research	519-766-4668	murray@halltechaquatic.com
Halliburton	David	Environment Canada	819-953-1128	dave.halliburton@ec.gc.ca
Hamilton	Andrew	Commission Environmental Cooperation	613-523-3345	Andrewlhamilton@rogers.com
Harding	Katie	Trent University	705-748-0922	katieharding@trentu.ca
Hardy	David	Canmet	613-992-0029	dhardy@nrcan.gc.ca
Hare	Landis	Université du Québec	418-654-2640	landis@inrs-ete.uquebec.ca
Harris	Gary	Business	902 757 0232	hits@ns.sympatico.ca
Harris	Peter	Environment Canada	819-953-1673	Peter.Harris@ec.gc.ca
Harttrup	Nancy	Stantec Consulting	519-836-6050	nharttrup@stantec.com
Harwood	Manon	Environment Canada	514-496-7106	manon.harwood@ec.gc.ca
Hawkins	Stephanie	Indian Northern Affairs Canada	867-975-4555	hawkinss@inac.gc.ca
Haya	Kats	Dept. of Fisheries and Oceans	506-529-5916	hayak@mar.dfo-mpo.gc.ca
Hebert	Craig	Environment Canada	819-998-6693	craig.hebert@ec.gc.ca
Hedley	Kathleen	Environment Canada	819-953-1553	kathleen.hedley@ec.gc.ca
Helbing	Caren	University of Victoria	250-721-6146	chelbing@uvic.ca
Hendershot	William	Mcgill University	514-398-7942	William.Hendershot@mcgill.ca
Hewitt	Mark	Environment Canada	905-319-6924	mark.hewitt@ec.gc.ca
Hilderman	Murray	SERM	306-787-6181	mhilderman@serm.gov.sk.ca
Hodson	Peter	Queen's University	613-533-6129	hodsonp@biology.queensu.ca
Hontela	Alice	University of Lethbridge	403-394-3929	alice.hontela@uleth.ca
Hruska	Kimberly	Environment Canada	306-975-5739	kim.hruska@ec.gc.ca
Hughes	Meg	EVS Environment Consultants	604-986-4331	mhughes@evsenvironment.com
Huras	Craig	ASI Group Ltd.	905-641-0941	churas@asi-group.com
Ikonomou	Michael	Dept.of Fisheries and Oceans	250-363-6804	ikonomoum@dfo-mpo.gc.ca
Ings	Jennifer	University of Guelph	519-824-4120	jings@uoguelph.ca
Innes	Elizabeth	Health Canada	613-952-9745	elizabeth_innes@hc-sc.gc.ca
Jackson	Francis	Indian Northern Affairs Canada	867-669-2666	JACKSONF@INAC.GC.CA
Jackson	Stéphanie	Environment Canada	819-956-9849	stephanie.jackson@ec.gc.ca
Jefferson	Catherine	Can. Water Waste Water Assoc.	705-652-9732	rpipiens@nexicom.net

Jiapizian	Paul	Environment Canada	819-953-6700	paul.jiapizian@ec.gc.ca
Johnston	Blair	University of Aberdeen	44-1224-272396	blair.johnston@abdn.ac.uk
Jones	Chris	Ontario Ministry of Environment	705-766-1724	chris.jones@ene.gov.on.ca
Jordan	Bonna	Environment Canada	413-523-2621	bonna.jordan@ec.gc.ca
Kaminski	Gregory	Health Canada	613-736-3687	greg_kaminski@hc-sc.gc.ca
karalis	Elpi	Environment Canada	819-994-2307	elpi.karalis@ec.gc.ca
Khan	Colin	Queen's University	613-533-6000	khanc@biology.queensu.ca
Khan	Haseen	Newfoundland Environment	709-729-2535	hkhan@gov.nl.ca
Kidd	Karen	Dept. of Fisheries and Oceans	204-983-5226	kiddk@dfo-mpo.gc.ca
Kierstead	Ted	Pollutech Enviroquatics Ltd.	519-339-8787	tkierstead@peql.net
Kightley	Meghan	Goldcorp Inc.	807-735-2077	mkightley@goldcorp.com
Kilgour	Bruce	Jacques Whitford Environ. Ltd.	613-738-0708	bkilgour@jacqueswhitford.com
King	Morgan	Natural Resources Canada	613-992-9431	moking@nrcan.gc.ca
Kirsch	Penny	Enviro-Test Laboratories	613-731-1005	pkirsch@envirotest.com
Kojomkulov	Eric	Kumtor Operating Company	306-956-6200	eric_kojomkulov@kumtor.com
Kovacs	Tibor	Paprican	514-630-4101	tkovacs@paprican.ca
Kraemer	Lisa	Université du Québec	418-654-2583	lisa_kraemer@inrs-ete.uquebec.ca
Kroetsch	Jim	Ch2m Hill	519-579-3500	jkroetsc@ch2m.com
Kruper	Nancy	Environment Canada	780-435-7307	nancy.kruper@ec.gc.ca
Kussatz	Carola	Uba	49-30-8903-4169	carola.kussatz@uba.de
Lacroix	Alexandra	Universite du Quebec a Montreal	514-987-3000	alexandralacroix@hotmail.com
Laforet	Scott	Streampoint Solutions	416-740-1862	scott@ws.ca
Lafortune	Anne-Marie	CEAEQ, MENV	418-643-1301	anne-marie.lafortune@menv.gouv.qc.ca
Lander	Lesley	Environment Canada	819-953-1700	lesley.lander@ec.gc.ca
Larose	Celine	Captial Regional District	250-360-3144	clarose@crd.bc.ca
Lawuyi	DR. Richard	Environment Canada	819-953-6226	Richard.Lawuyi2@ec.gc.ca
Lean	David	Ottawa University	613-562-5800	dlean@science.uottawa.ca
LeDrew	Beverly	Health Canada	613-941-5600	Beverly_LeDrew@hc-sc.gc.ca
Lee	Lucy	Wilfrid Laurier University	519-884-0710	llee@wlu.ca
Lee	Sung-kyu	Korea Institute of Toxicology	82 42 860 7466	sungklee@kitox.re.kr
Leonard	Jim	Dept. of Fisheries and Oceans	902-426-8880	leonardj@mar.dfo-mpo.gc.ca
Lewis	Andrew	Greater Vancouver Regional Dist.	604- 451-6632	Andrew.Lewis@gvrd.bc.ca
Linquist	Janice	N.a.r. Environ. Consultants Inc.	705-522-5990	nar@cyberbeach.net
Lister	Andrea	University of Guelph	519-824-4120	alister@uoguelph.ca
Lo	Amy	Domtar, Inc.	613-938-4585	amy.lo@domtar.com
Lopez	Mirey	Environment Canada	780 -951-8617	mirey.lopez@ec.gc.ca
Lorenzen	Angela	Agriculture Agri-Food Canada	519-457-1470	lorenzena@agr.gc.ca
Lowell	Richard	Environment Canada	306-975-6303	rick.lowell@ec.gc.ca
Macey	Debbie	CAEAL	613-233-5300	dmacey@caeal.ca

MacGregor	Don	Environment Canada	613-990-9540	Don.MacGregor@ec.gc.ca
Maddison	Linda	Environment Canada	819-953-5046	Linda.Maddison@ec.gc.ca
Maertens	Rebecca	University of Ottawa	613-957-3135	rebecca_maertens@hc-sc.gc.ca
Maher	Sue	Newfoundland Dept.of Environ.	709-729-2273	smaher@mail.gov.nl.ca
Maisonneuve	France	Environment Canada	613-998-8088	france.maisonneuve@ec.ga.ca
Martel	Pierre	Paprican	514-630-4101	pmartel@paprican.ca
Masson Stogran	Deborah	SGS Lakefield Research	705-652-2041	dstogran@lakefield.com
Matteau	Isabelle	Environment Canada	514-283-6216	isabelle.matteau@ec.gc.ca
McCann	Janet	University of Waterloo	519-888-4567	jhmccann@sciborg.uwaterloo.ca
McGeer	Jim	Natural Resources Canada	613-947-3451	jmcgeer@nrcan.gc.ca
Mckeown	Derek	Hoskin Scientific	613-354-0540	dmckeown@hoskin.ca
McMaster	Mark	Environment Canada	905-319-6906	Mark.McMaster@ec.gc.ca
Mcrae	Terence	Environment Canada	819-953-4007	mcrae.terence@ec.gc.ca
Metcalfe	Chris	Trent University	705-748-1011	cmetcalfe@trentu.ca
Middelraad	Irene	D.G. Dixon & Associates	519-767-0842	imiddelraad@hotmail.com
Miettunen	Anita	Environment Canada	819-953-1674	anita.miettunen@ec.gc.ca
Miller	Jennifer	Miller Environmental	705-431-7733	miller.smith@sympatico.ca
Mills	Carole	Indep. Environ. Monitor. Agency	867-669-9141	monitor@yk.com
Mimeault	Caroline	University of Ottawa	613-562-5800	cmimeaul@science.uottawa.ca
Minnery	Sarah	University Of Ottawa	613-562-5800	sminnery@hotmail.com
Misra	Ravindra	R.M.L.A.University	905-293-9561	misra_ravindra@yahoo.ca
Moody	Mary	Saskatchewan Research Council	306-933-5469	moody@src.sk.ca
Moon	Thomas	University of Ottawa	613-562-5800	tmoon@science.uottawa.ca
Moore	Dan	Alberta Newsprint	708-778-7029	danm@albertanewsprint.com
Moore	Jennifer	Environment Canada	819-997-5674	jennifer.moore@ec.gc.ca
Moran	Tim	Pollutech Enviroquatics Ltd.	519-339-8787	tmoran@pollutechgroup.com
More	Robert	Environment Canada	604-666-8329	bob.more@ec.gc.ca
Munger	Steve	Can. Nuclear Safety Commission	613-947-2973	mungers@cnsc-ccsn.gc.ca
Munro	Scott	Sarnia-Lambton Environ. Assoc.	519-332-2010	scottmunro@sarniaenvironment.com
Munson	Barry	Environment Canada	780-951-8833	barry.munson@ec.gc.ca
Murray	Janine	Environment Canada	819-997-4070	janine.murray@ec.gc.ca
Nadeau	Jennifer	Natural Resources Canada	613-992-1762	jenadeau@NRCan.gc.ca
Nagpal	Narender	Government of British Columbia	250-387-9507	Narender.Nagpal@gems5.gov.bc.ca
Nero	Vincent	University of Waterloo	519-747-7187	vnero17@hotmail.com
Ng	Robert	Greater Vancouver Regional Dist.	604-451-6012	Robert.Ng@gvrd.bc.ca
Niimi	Arthur	Dept. of Fisheries and Oceans	905-336-4868	niimia@dfo-mpo.gc.ca
Niyogi	Som	McMaster University	905-525-9140	niyogis@mcmaster.ca
Norwood	Warren	University of Waterloo	905-336-4694	warren.norwood@ec.gc.ca
Nowierski	Monica	Environment Canada	905-336-4405	Monica.Nowierski@ec.gc.ca

Olivier	Sarah	Environment Canada	819-994-9484	sarah.olivier@ec.gc.ca
Orr	Patti	Minnow Environmental Inc.	905-567-8771	porr@minnow-environmental.com
Orvoine	Jord	Inrs Ete	418-654-2583	jordbreizh@hotmail.com
Osmond	Dave	Gartner Lee Ltd.	604-299-4144	dosmond@gartnerlee.com
Ostapyk	Kim	Health Canada	613-952-9740	kim_ostapyk@hc-sc.gc.ca
Paine	Michael	Paine, Ledge & Associates	604-924-8126	michael_paine@telus.net
Palace	Vince	Dept. of Fisheries and Oceans	204-983-5004	palacev@dfo-mpo.gc.ca
Papineau	Joanne	Environment Canada	819-997-2721	joanne.papineau@ec.gc.ca
Paradis	Sylvain	Dept. of Fisheries and Oceans	613-993-0602	paradiss@dfo-mpo.gc.ca
Parent	Lise	Télé-université	514-840-2747	lparent@teluq.uquebec.ca
Park	Brad	Dept. of Fisheries and Oceans	204-984-5542	parkb@dfo-mpo.gc.ca
Pasternak	John	Environment Canada	604-666-8077	john.pasternak@ec.gc.ca
Paterson	Renee	Newfoundland Dept. of Environ.	709-729-1159	reneepaterson@gov.nl.ca
Pauli	Bruce	Environment Canada	613-998-6690	bruce.pauli@ec.gc.ca
Pellerin	Jocelyne	ISMER-UQAR	418-723-1986	jocelyne_pellerin@uqar.qc.ca
Peramaki	Lissa	Dept. of Fisheries and Oceans	613-998-1446	peramakil@dfo-mpo.gc.ca
Perez	Barbara	University of Ottawa	819-561-9576	barbaraperez25@hotmail.com
Phommavong	Thon	Saskatchewan Environment	306-787-9986	tphommavong@serm.gov.sk.ca
Pickard	Janet	BC Research	604-224-4331	jpickard@bcresearch.com
Plant	Wes	Environment Canada	416-739-4272	Wesley.Plant@ec.gc.ca
Playle	Richard	Wilfrid Laurier University	519-884-0710	rplayle@wlu.ca
Poirier	David	Ontario Ministry of Environment	416-235-6362	dave.poirier@ene.gov.on.ca
Porebski	Linda	Environment Canada	819-953-4341	Linda.Porebski@ec.gc.ca
Portt	Cam	C. Portt & Associates	519-824-8227	cportt@sentex.net
Postlethwaite	Emma	Environment Canada	819-997-7091	emma.postlethwaite@ec.gc.ca
Prairie	Robert	Noranda	514-630-9357	prairie@ntc.noranda.com
Princz	Juliska	Environment Canada	613-990-9544	Juliska.Princz@ec.gc.ca
Providenti	Miguel	Carleton University	613-520-2600	miguelprovidenti@pigeon.carleton.ca
Quinn	Brian	Environment Canada	514-496-7104	brian.quinn@ec.gc.ca
Ralitsch	Milan	Enviro-Test Laboratories	613-731-1005	milan@envirotest.com
Ramachandran	Shahunthala	Queen's University	613-533-6000	devis@biology.queensu.ca
Rawn	Gary	Dept. of Fisheries and Oceans	613-993-3065	rawng@dfo-mpo.gc.ca
Regan	Christine	University of Guelph	519-824-4120	cregan@uofguelph.ca
Renoux	Agnès	Sanexen Environ. Services Inc.	450-652-9990	arenoux@sanexen.com
Ribey	Sandra	Environment Canada	819-953-3456	sandra.ribey@ec.gc.ca
Rickwood	Carrie	Environment Canada	306-975-4655	carrie.rickwood@ec.gc.ca
Robidoux	Pierre Yves	National Res. Council of Canada	514-283-6447	Pierre-Yves.Robidoux@cnrc-nrc.gc.ca
Robinson	Peter	Environment Canada	819-994-3714	Peter.Robinson@ec.gc.ca
Rodrigue	Danielle	Environment Canada	819-953-1522	danielle.rodrigue@ec.gc.ca

Roe	Susan	Environment Canada	819-994-8405	susan.roe@ec.gc.ca
Ross	Gregory	Queen's University	613-533-6938	rossg@post.queensu.ca
Rouette	Marie-eve	Lab-bell	819-539-8508	merouette@lab-bell.com
Ruby	Sylvia	Concordia University	514-848-3428	sruby@alcor.concordia.ca
Rudnitski	Kristina D.	Environment Canada	819-953-7944	kristina.rudnitski@ec.gc.ca
Russel	Cynthia	Minnow Environmental Inc.	905-567-8771	crussel@minnow-environmental.com
Russo	Nick	Environment Canada	604-666-1092	Nick.Russo@ec.gc.ca
Rutherford	Les	Environment Canada	902-426-2295	les.rutherford@ec.gc.ca
Ryan	Michael	Dept.of Fisheries and Oceans	204-775-2762	ryanmj@dfo-mpo.gc.ca
Salazar	Michael	Applied Biomonitoring	425-823-3905	appbio@comcast.net
Sanderson	Hans	University of Guelph	519-824-4120	hsander@uoguelph.ca
Schetagne	Roger	Hydro-Québec	514-289-2211	schetagne.roger@hydro.qc.ca
Schirmer	Kristin	UFZ, Leipzig, Germany	49-341-235-2699	kristin.schirmer@uoe.ufz.de
Schnabel	Sabine	Environment Canada	819-953-9526	sabine.schnabel@ec.gc.ca
Schneider	Kim	University of Guelph	519-836-6050	kschne01@uoguelph.ca
Schneider	Uwe	Environment Canada	819-953-8599	uwe.schneider@ec.gc.ca
Schroeder	Julie	Ontario Ministry of Environment	416-314-9169	schroeju@ene.gov.on.ca
Schudoma	Dieter	Federal Environmental Agency	49-30 89032195	dieter.schudoma@uba.com
Schwartz	Melissa	Natural Resouces Canada	613-947-7383	mschwart@NRCan.gc.ca
Scroggins	Rick	Environment Canada	613-990-8569	scroggins.richard@etc.ec.gc.ca
Sebastian	Mary	University of Windsor	519-253-3000	mparekunnel@hotmail.com
Sferrazza	John	ASI Group Ltd.	905-641-0941	john@asi-group.com
Sheppard	Steve	Ecomatters Inc.	204-753-2747	sheppards@ecomatters.com
Sherry	Jim	Environment Canada	905-336-4813	jim.sherry@cciw.ca
Siah	Ahmed	Ismer	418-722-1967	ahmed_siah@uqar.qc.ca
Simmons	Denina	Trent University	705-748-1011	deninasimmons@trentu.ca
Siwik	Paula	Environment Canada	780-951-8824	paula.siwik@ec.gc.ca
Solomon	Keith	University of Guelph	519-837-3320	ksolomon@uoguelph.ca
Somers	Jim	Standards Council of Canada	613-238-3222	jsomers@scc.ca
Somers	Keith	Ontario Ministry of Environment	705-766-2408	somers@zoo.utoronto.ca
Sotornik	Dana	University of Waterloo	519-622-4785	soto2010@mach1.wlu.ca
Spiers	Graeme	Laurentian University	705-675-1151	gspiers@laurentian.ca
Spry	Doug	Environment Canada	819-953-3206	doug.spry@ec.gc.ca
Squires	Allison	University of Saskatchewan	306-966-7441	ajs023@mail.usask.ca
St. Jean	Sylvie	Environment Canada	506-851-3195	sylvie.st-jean@ec.gc.ca
Steeves	Troy	Environment Canada	506-851-3837	Troy.Steeves@EC.GC.CA
Stephenson	Gladys	Stantec Consulting Ltd	519-836-6050	gstephenson@stantec.com
Suffredine	Lori	Environment Canada	604-666-2880	lori.suffredine@ec.gc.ca
Sunahara	Geoffrey	National Res. Council of Canada	514-496-8030	geoffrey.sunahara@nrc.ca

Swain	Les	BC Min. Water, Land Air Protect.	250-387-4227	Les.Swain@Gems7.gov.bc.ca
Taillefer	David	Environment Canada	819-956-5225	david.taillefer@ec.gc.ca
Taylor	Lisa	Stantec Consulting Ltd.	519-763-4412	lntaylor@stantec.com
Taylor	Darrell	NS Dept. Environment Labour	902-424-2570	taylordl@gov.ns.ca
Taylor	Ken	Environment Canada	819-953-3976	Ken.Taylor@ec.gc.ca
Taylor	Jacqueline	Tetres Consultants Inc.	204-942-2505	jtaylor@tetres.ca
Temple	John	Environment Canada	819-994-6245	jtemple@magma.ca
Tetreault	Gerald	Environment Canada	905-319-6998	Gerald.Tetreault@ec.gc.ca
Toker	Carmen	Maxxam Analytics Inc.	780-468-3508	ctoker@edm.maxxam.ca
Toner	Glen	Carleton University	613-520-2600	glentoner@aol.com
Topp	Edward	Environment Canada	519-457-1470	toppe@agr.gc.ca
Topping	Paul	Environment Canada	819-953-0663	paul.topping@ec.gc.ca
Trew	David	Alberta Environment	780-427-3029	david.trew@gov.ab.ca
Trottier	Sylvain	Environment Canada	514-283-2460	sylvain.trottier@ec.gc.ca
Trudeau	Vance	University of Ottawa	613-562-5800	vtrudeau@science.uottawa.ca
Trudeau	Suzanne	Environment Canada	613-998-9475	suzanne.trudeau@ec.gc.ca
Trudel	Ken	SL Ross Environ. Research Ltd	613-232-1564	Ken@slross.com
Trudel	Lise	Environment Canada	819-953-1527	lise.trudel@ec.gc.ca
Truelson	Robert	Govement of Yukon	867-667-3217	bob.truelson@gov.yk.ca
Turcotte	Dominique	Queen's University	613-533-6000	domini@chem.queensu.ca
van Aggelen	Graham	Environment Canada	604-924-2513	graham.vanaggelen@ec.gc.ca
Van Der Vliet	Leana	Environment Canada	819-953-3197	leana.vandervliet@ec.gc.ca
Van Poppelen	Paul	Greater Vancouver Regional Dist.	604-451-6053	paul.van.poppelen@gvrd.bc.ca
Varao	Adrian	ASI Group Ltd.	905-641-0941	avarao@asi-group.com
Veilleux	Stephan	Bodycote Essais De Mat. Can.	418-871-8722	veilleux.s@bodycote.ca
Verburg	Jason	Bruce Power	519-361-2673	jason.verburg@brucepower.com
Vigneault	Bernard	Natural Resources Canada	613-947-8763	Bernard.Vigneault@nrcan.gc.ca
Voss	Ron	Paprican	514-630-4126	rvoss@paprican.ca
Walker	Sherry	Environment Canada	819-953-1571	sherry.walker@ec.gc.ca
Wallace	Angela	University of Waterloo	905-336-4694	angela.wallace@ec.gc.ca
Watson	Glen	Inco Ltd.	705-682-8231	gwatson@inco.com
Watson-Leung	Trudy	Ontario Ministry of Environment	416-235-6363	watsontr@ene.gov.on.ca
Whall	John	Health Canada	613-736-3868	john.david.whall@hc-sc.gc.ca
Wickstrom	Geoff	Keystone Environmental Ltd.	604-430-0671	gwickstrom@keystoneenviro.com
Wilson	Christian	University of Guelph	519-824-4120	christia@uoguelph.ca
Windle	Wiletta	Environment Canada	819-953-9974	wiletta.windle@ec.gc.ca
Winter	Anna	Wilfrid Laurier University	519-884-0710	annaraewint@netscape.net
Wisniewski	Wendy	Inco Ltd.	705-682-8247	wwisniewski@inco.com
Woodhouse	Amanda	University of Ottawa	613-562-5800	mwood785@science.uottawa.ca

Wren	Chris	C. Wren and Associates	519-766-1039	cwren@cwren.com
Wright	Margaret	Dept. of Fisheries and Oceans	250-756-7269	wrightma@dfo-mpo.gc.ca
Wytrykush	Carla	University of Windsor	519-253-3000	wytryku@uwindsor.ca
Young	Iris	Department of National Defence	819-994-1681	young.ij@forces.gc.ca
Zajdlik	Barry	Zajdlik & Associates	519 856-9440	bzajdlik@sentex.net
Zaranko	Danuta	ZEAS	519-836-9176	dzaranko@sentex.net
Zosimadis	Mike	Streampoint Solutions	416-740-1862	mike@streampoint.biz

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The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings may be available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

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